BIOCHEMICAL RESPONSE OF ACTIVATED SLUDGE PROCESSES TO HYDRAULIC, pH AND TEMPERATURE SHOCK LOADS

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Scope and Method of Study: The biochemical response of a continuous flow completely mixed activated sludge system to shock loadings consisting of changes in dilution rate, pH and temperature was investigated using glucose as the organic carbon source. Changes in dilution rate were studied under two different conditions, i.e., with constant influent organic concentration and constant daily organic loading. The effect of changes in reactor pH was investigated under conditions in which cells were recycled, as well as in "one pass" flow-through systems. The response to changes in temperature of the mixed liquor in the aerator was studied at two different reactor detention times, eight hours and four hours.

The major parameters studied included biological solids concentration, effluent COD, anthrone COD, cell protein and carbohydrate. In a number of experiments the RNA and DNA content of the cells were determined. In a few experiments volatile acids released into the medium were determined by gas-liquid chromatography and carbon, nitrogen and hydrogen content of the sludge was determined.

Findings and Conclusions: In all cases of shock loadings causing a severe reactor response wherein the system tended to recover, the effluent COD reached a peak value during the transient and then receded to a new steady state value. At the same time the biological solids concentration in the reactor reached a low value, and then rose to a new steady state value. It was observed that a reactor operating at a detention time of eight hours has considerable ability to accommodate hydraulic shock loads.

The yield of cells varied more under constant organic loading conditions of hydraulic shock loading than under constant concentration conditions. Under both conditions of operation an increase in dilution rate caused a lowering of the protein content and increase of the carbohydrate content of the sludge.

Shock loading involving a decrease in pH caused a change in microbial predominance from rod-shaped bacteria to filamentous types. Also, it resulted in a decrease in protein content of the sludge and an increase in the carbohydrate content. Even though these systems showed a severe transient state response, the few cells which were retained in the reactor were able to gain acclimation and recover from pH shock loading to reactor pH values as low as 3.00.

Cold temperature shock loadings were observed to cause more damage to a system operating initially at 25^{o}C than did the hot temperature shock loadings. Acetic acid was one of the prominent metabolic products in the medium during some temperature shock experiments.

ADVISER'S APPROVAL_____

BIOCHEMICAL RESPONSE OF ACTIVATED SLUDGE PROCESSES TO HYDRAULIC, pH AND TEMPERATURE SHOCK LOADS

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CHAPTER I

INTRODUCTION

A. General

Since the dawn of humanity, man has been in search of pure water for sustenance and recreation. During the early days the problem was solved by moving of settlements rather than by attempting to purify the waters available at a given site. As communities became larger and the quality of available supplies began to deteriorate, treatment to purify the waters began. As industrial development demanded larger and larger quantities of pure waters, and larger quantities of waste waters were produced, the problem of pollution of the aqueous environment was aggravated. In order to protect streams from pollution, several treatment processes were developed to improve the quality of the waste waters before they were discharged into receiving streams.

An assessment of the magnitude of the water pollution problem must take into account the total available supply of water and the quantity of waste waters. In 1954 in the USA the withdrawals of stream water for community and industrial use amounted to a third of the average annual streamflow. One-fifth of this amount was returned to streams as waste waters. In the year 2000, it is predicted

that withdrawals will be four-fifths, and polluted returns will be two-thirds of the entire streamflow (1).

The magnitude of the industrial wastes problem is becoming dangerously critical; a seven-fold increase is expected in the quantity of industrial wastes produced by the end of this century. These wastes as well as domestic wastes will have to be treated to avoid water pollution.

The treatment of waste waters has usually been considered to be, for the most part, a non-productive expenditure in the economy of the community or the industry, and the most inexpensive measures are usually employed. In the stabilization of the putrescible organic matter, biological treatment is used more than any other method developed thus far. The activated sludge process, developed over half a century ago, has become one of the prime processes in biological treatment. During the last two decades, "completely mixed" aeration chambers have come into vogue and have attracted the attention of many research workers in the water pollution control field.

Introduction of industrial wastes may cause "shock loading" problems in a complete mixing plant treating domestic sewage and the study of shock loads in relation to system response is of paramount importance in evaluating economical and biochemical feasibility of combined treatment of wastes of varying character.

B. Objectives of this Research

During recent years, shock loads involving a change

in the quality or quantity of the organic matter in the waste have been the subject of study in the bioenvironmental engineering laboratories at Oklahoma State University, Stillwater, Oklahoma. In order that the understanding of the basic patterns of response may be complete, investigation of shock loads involving changes in flow rate, pH and temperature seemed warranted. Such changes in the external environment would appear to be as important as changes in quantity and chemical nature of the carbon source. Transient state response in complete mixing systems cannot be kinetically defined in the absence of quantitative data. Even though attempts have been made by some workers to define transient state kinetics, they have not completely delineated the biological response mechanisms that operate during the application of a shock load. The present studies were designed to gain an insight into transient response of a completely mixed activated sludge system under such changes in external environment as flow rate (dilution rate), pH and temperature.

The shock loads under investigation include a change in flow rate (hydraulic shock) with constant influent organic concentration and with constant daily organic loading, changes in pH toward the acidic and alkaline ranges with "one pass" flow-through and under conditions of cell recycle to the reactor, and increases and decreases in temperature. The parameters examined before and during the resultant transient response include chemical oxygen demand (COD);

biological solids concentration; protein, carbohydrate, ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) content of the sludge; carbon, nitrogen, and hydrogen content of the biological solids; assessment of the metabolic intermediates released during the transient state; and observations of any gross changes in microbial predominance.

CHAPTER II

REVIEW OF LITERATURE

A. Historical Development of the Activated Sludge Process

The activated sludge process, developed over fifty years ago, has been primarily used for treatment of domestic wastes. In the past, the aeration tank has been designed essentially as a plug flow reactor. Aeration solids concentrations in the reactor were usually maintained at 2000-3000 mg/l and low loading rates (35 lbs/1000 cu ft) were employed. In attempts to reduce operating costs of the conventional process, several modifications have been devised. During the last two decades, the process has been employed either for combined or separate treatment of industrial wastes.

The conventional activated sludge process has several limitations as listed by Sawyer (2):

1. Biochemical oxygen demand (BOD) loadings are limited to 35 lbs/1000 cu ft of aeration volume.
2. There is a high initial oxygen demand.
3. There is a tendency for bulking of sludge.
4. The process does not produce an intermediate quality of effluent.
5. Recirculation is high for high BOD wastes.

6. Suspended solids load on the final sedimentation tank is high.

7. Oxygen or air requirements are high.

Busch and Kalinske (3) have cited ideal conditions for optimum activity in the activated sludge process; these are listed below:

1. Provision of a young flocculent sludge in the logarithmic stage of growth.

2. Maintenance of log-growth by controlled wastage of sludge.

3. Continuous loading of the organisms.

4. Elimination of anaerobic conditions at any point in the oxidative treatment.

Over the years, various modifications of the conventional process have been developed in an attempt to provide better purification or reduce costs. These include tapered aeration, step aeration, bio-sorption, the Kraus process, complete mixing and extended aeration or total oxidation. These developments are the outcome of operational research in prototype reactors and pilot plants as well as in laboratory units. The demand for treatment of industrial wastes also played a major part in the development of improved processes. Organic loadings as high as 400 lbs of BOD per 1000 cu ft of aerator volume per day could be successfully handled through modified processes of flow and aeration (4).

Step aeration was developed by Gould (5) at the Tall-

man Island plant in New York City. The process consisted of applying the waste stream in split fractions along the length of the aeration tank. The process was aimed at a uniformity in oxygen demand as the waste flows through the tank. The advantages claimed for the process include higher allowable BOD loadings, shorter detention times, and good settling characteristics of the sludge. However, it is doubtful whether this process can successfully respond to shock loads of the type under investigation in this study.

In contrast to step aeration, a process in which the oxygen supply is tapered along the length of the aeration tank (conforming approximately to the course of consumption of the carbon source in a conventional plug flow system) is known as "tapered" aeration. The major difference between this process and "step" aeration is that the control is effected on the air supply in the former, and the waste feed in the latter. This process may be suitably altered to provide a better response to shock loads in which the point of maximum oxygen uptake is shifted downstream along the activated sludge tank. Tapered aeration was first conceived by Kessler, Rohlich, and Smart (6).

Studies conducted by Setter (7) revealed that to produce an intermediate quality effluent, a modified aeration process employing low concentrations of mixed liquor suspended solids (300-600 mg/l) and a short aeration time (1.5-2 hours) could be practised. This process is operated at approximately the same range of solids concentration

employed in the studies reported in this dissertaion. (However, there are other differences, e.g., complete mixing, sludge age, and detention time).

Kraus (8) developed a system of operation generally known as the Kraus process for permitting high BOD loadings and improving sludge settling properties. The process employs a nitrification tank in which part of the return sludge is aerated with a mixture of digester supernatant and digested sludge. There is some doubt as to whether the process is capable of treating quantitative (organic) shock loads, since the active mass of micro-organisms in the return sludge is not likely to be more than in a normal activated sludge. However, provision of a secondary aeration tank may prevent the development of anaerobic conditions in the return sludge.

The bio-sorption process employed the "adsorptive" properties of the activated sludge and was developed by Ulrich and Smith (9). In this process, activated sludge, well-conditioned by reaeration, is brought into contact with the raw waste water under intense aeration for a period of thirty minutes. The rapid removal of waste BOD is often attributed to the phenomenon of adsorption, which may be applicable to colloidal matter but is of questionable validity for dissolved organic solids, especially in the light of the findings of Gaudy and Engelbrecht (10), who have reported that the process is biochemical in nature as regards soluble organic materials.

The high rate activated sludge process was developed to treat high organic loadings at high mixed liquor suspended solids levels (4,000-10,000 mg/l) and low detention times (1-2 hours) in the aerator. Pasveer (11) found in his laboratory studies that it was possible to accomplish complete nitrification at BOD loadings of 112 lbs per 1000 cu ft per day and BOD removals of 93 per cent could be achieved at loadings as high as 3750 lbs per day per 1000 cu ft. However, practical development of the process resulted only after the perfecting of high capacity aeration devices.

Extended aeration systems were developed to provide a treatment which could minimize the sludge disposal problem and obviate the need for anaerobic sludge digestion. High detention times (24 hours) in the aerator are employed and the process is used primarily for small installations operated without extensive laboratory control. Due to increased aeration periods, the effluent is highly nitrified.

Although the previously listed modifications may represent improvements on the originally conceived conventional activated sludge process, they were, for the most part, designed to operate as plug flow reactors and they did not in general provide intimate mixing of the waste with the micro-organisms in all parts of the aeration tank. During the past two decades, the geometric design of aeration tanks has been gradually changing to one which fosters complete and rapid mixing of the incoming wastes with the mixed liquor in the tank. According to McKinney (12), the com-

plete mixing aeration unit acts as a surge tank to level out wide fluctuations in organic load. The use of the entire mass of the activated sludge to stabilize the organic matter allows better utilization of the air supplied.

Tenney, et al. (13) state that the complete mixing activated sludge process can operate successfully with high waste loadings and fairly young cells.

Preventing short-circuiting of flow from inlet to outlet is very important in the establishment of complete mixing conditions. This can usually be achieved by efficient mixing devices. In this connection, intense aeration serves the dual purpose of providing air for biological oxidation and agitation for rapid and complete mixing.

Completely mixed systems have been used for treatment of various types of organic wastes from industries. The following examples show the varied range and wide application of this type of design in the treatment of trade effluents.

Petroleum wastes were treated in bench scale units by Coe (14). He indicated that treatment efficiencies of 90-95 per cent could be obtained at organic loadings of 140 lbs per 1000 cu ft per day.

Taylor (15) employed a uniformly mixed aerator to treat wastes from the manufacture of orlon. The waste was successfully treated at BOD loadings of 0.4 lbs per lb of mixed liquor suspended solids per day. The system was exposed to pH values of 4.5-10.5 without seriously upsetting

treatment efficiency.

Hoover and Porges (16) studied treatment of dairy wastes in a completely mixed system. They observed that 50 per cent of the waste was oxidized and 45-48 per cent was assimilated.

Quirk, et al. (17), using laboratory batch activated sludge units, found that board-mill wastes could be successfully treated by the activated sludge process.

Industrial wastes treated by activated sludge units are discussed more fully in following sections reviewing the effects of changes in dilution rate, pH, and temperature on continuous flow reactors.

From the foregoing review, it is evident that, until recently, there has been very little direct information available on the response of complete mixing systems to shock loads. Gaudy (18), in his study of qualitative shock loads, observed that successful response depended on the availability of a readily available nitrogen source and on culture age. He also observed that acclimation to one substrate may confer acclimation to another substrate.

Komolrit (19) studied the effect of qualitative shock loading, using continuous flow completely mixed bench scale units and found that the ability to respond to such a shock load was dependent on dilution rate, biological solids concentration, sludge activity, and availability of nitrogen in the waste water.

Krishnan (20) investigated the biochemical effects of

quantitative shock loads using a continuous flow unit with and without cell recycle. He found that successful response depended on the dilution rate, availability of adequate nitrogen in the waste and solids recycle. He also found that acetic acid was released as a metabolic intermediate in systems without cell recycle and at high dilution rates.

The succeeding portions of the literature review are devoted to work bearing on the effects of changes in dilution rate, pH and temperature on the operation of fluidized microbial cultures since these are the parameters studied in the present investigation.

B. Effect of Dilution Rate and Loading on the Performance of Continuous Cultures

Since the concept of complete mixing in activated sludge is an idea borrowed from microbiology, it seems apparent that a review of literature pertaining to continuous culture of pure and mixed populations should provide useful information. From the theory of complete mixing, it is well established that under steady state conditions the logarithmic growth rate of the culture is determined by (and equal to) the dilution rate of the system. Hence a review of the effect of dilution rate on metabolic response is included in this section.

The dependence of cellular composition on the growth rate or dilution rate has been investigated by research workers using pure cultures. The main interest has centered around changes of the RNA and DNA content of the cells.

Both constituents are vital to microbial growth; it is known that the RNA content can vary widely, but the DNA content is fairly constant.

Herbert (21, 22) stressed the relationship between RNA and DNA content and the specific growth rate of the system. He showed that the RNA content increased with the dilution rate during continuous cultivation of Aerobacter aerogenes, whereas the DNA decreased somewhat. He found in similar experiments with Staphylococcus aureus that the RNA content appeared to be a linear function of the growth rate, whereas with Bacillus megaterium, a plot of the RNA content versus dilution rate was not linear. Also, the protein percentage was found to decrease with dilution rate.

Jeener (23) studied synthesis of RNA and protein in continuous cultivation, with a strain of Polytomella caeca. He observed that the RNA to protein ratio varied with the dilution rate, which did not agree with the assumption that the protein content of the cell was a function of its RNA content.

Schaechter, Maaloe, and Kjeldgaard (24), using continuous cultures of Salmonella typhimurium, found that the amount of RNA and DNA can be expressed as an exponential function of specific growth rate. It was further indicated that the amount of protein synthesized per cell per unit of time was approximately proportional to the RNA content of the cells for a wide range of specific growth rates. There was a rapid buildup of cell mass and RNA following a rapid

increase in growth rate.

Neidhart and Magasanik (25) examined the formation of protein, RNA and DNA using two strains of Aerobacter aerogenes and confirmed the finding that there is a rapid increase in RNA synthesis during a transition tending to increase growth rate.

Holme (26) studied glycogen synthesis in Escherichia coli under nitrogen-limiting conditions at various dilution rates and found that glycogen synthesis increased with decreasing dilution rates and was not limited by nitrogen limitation in the reactor.

Wright and Lockhart (27) grew Escherichia coli in continuous culture in a defined medium at various dilution rates with either carbon or nitrogen as the rate limiting substrate. Average cell size and the cellular content of RNA, protein and free amino acids varied with both growth rate and the nature of the limiting nutrient, being greater at higher growth rates and greater for nitrogen-limited than for carbon-limited cultures.

The effect of dilution rate and loading on the performance of mixed cultures and activated sludge has been under investigation for some time. Cassel, Sulzer and Lamb (28) found that in a microbial mixed culture, dilution rate is a strong selective agent and that different species predominate at different dilution rates. This point is of considerable importance in the establishment of kinetic relationships and analysis of biochemical

changes occurring during growth. Their conclusions were based on observation of changes in pigmentation, changes in color of the mixed liquor, and changes in number of protozoa found in the mixed liquor.

Genetelli and Heukelekian (29), working with activated sludge concluded that, even at the same sludge loading ratio variations in dilution rate, solids concentration and applied BOD cause different responses by activated sludge. They observed in addition that at any particular sludge loading ratio, sludge yield increased as the detention time decreased.

Hopwood and Downing (30) noted in continuous flow experiments that "sludge growth index" (weight of sludge formed per unit weight of BOD applied) decreased progressively from 0.9 at a detention time of 2 hours to 0.38 at a detention time of 36 hours.

Tenney, et al. (13) found that in a system of complete mixing activated sludge at a solids retention time of 0.25 days (solids retention time = total weight of solids in a biological system/total weight of solids leaving the system/unit time), and volumetric organic loadings from 60 to 1690 lbs COD per day per 1000 cu ft, the fate of the input COD was as follows: 12 per cent in the effluent, 42 per cent lost (probably oxidized), and 46 percent converted to solids.

Ramanathan (31) working on the kinetics of completely mixed activated sludge, observed that the physiological

growth parameters (maximum growth rate μ_{max} and saturation constant k_s) obtained from batch experiments using seed from a continuous flow unit are dependent on the dilution rate at which the cells were growing before being used in the batch growth studies.

Komolrit (19) studied substrate interactions under shock loading conditions at various dilution rates. He concluded that the hydraulic loading rate indirectly controls the manifestation of substrate inhibition by controlling the growth of biological solids in the system.

Krishnan (20) observed that during quantitative shock loading conditions, the release of volatile acids in a glucose fed system was dependent on the dilution rate as well as the biological solids concentration in the aerator.

The foregoing review attests to the importance of dilution rate in determining the metabolic response in a completely mixed system. However, there have been some studies pertaining to hydraulic transients in a completely mixed system which have even more bearing on the current investigation. The complexity of the mathematical problems encountered in seeking kinetic expression for the unsteady state has been discussed by Reiner and Spiegelman (32).

Mason and Piret (33) using the Laplace transformation, developed general equations which could be used to describe a wide variety of transient conditions in continuous stirred tank reactor systems. These were claimed to be applicable to a variety of conditions involving reactors in

which first order reactions are being carried out.

Leudeking and Piret (34) developed steady state and transient equations for continuous flow fermentation processes. They found that experimental measurements of continuous lactic acid fermentations conducted at controlled pH levels agreed with the equations they developed for transient and steady states.

Storer (35) studied the kinetic properties of natural populations in a chemostat during transient conditions imposed by a change in the influent organic concentration. He concluded that the Monod equation is not valid during the transient state. He also noted the existence of a "growth-rate hysteresis" phenomenon during the transient state, as was postulated by Perret (36).

C. Effect of pH on Micro-organisms and Continuous Cultures

The effect of pH on microbial cultures has been the subject of research with pure cultures for a very long time. A brief review of the pertinent aspects which may be helpful in the analysis of the findings and data presented in this dissertaion is included herein.

Gale and Epps (37) studied the effect of pH on inducible enzymes in Escherichia coli. Certain amino acid deaminases showed optimum activity in the alkaline range of pH. On the other hand, amino acid decarboxylases which cause the production of amines resulting in a raising of pH toward neutrality were found only in acid media. The amino acid deaminases produce carboxylic acids tending to lower

the pH of the medium toward neutrality. These workers concluded that bacteria react to a change in their external environment in such a way that the resultant change is minimized.

Jordan and Jacobs (38) investigated the performance of Bacterium coli under acid conditions and constant food supply in a semi-continuous unit (continuous inflow of feed with no outflow, but culture volume was maintained constant by evaporation). Under these conditions, they observed that the cells functioned more efficiently at lower pH than higher pH as regards growth rate, viability, and food storage. This behavior is in striking contrast to the findings of Gale and Epps (37), who reported that many of the enzymes of Bacterium coli are sensitive to acid conditions.

The findings of Jordan and Jacobs (38) are in partial agreement with those of Joseph and Shay (39), who investigated the viability of Escherichia coli in acid mine waters. They found that some reduction in numbers occurred, but the tolerance to acid conditions was significant. The rate of reduction of viable count was rather rapid, but a small number survived the 24-hour testing period even at pH 2.0.

Gibson (40) observed that in the rumen of the sheep, protozoa were killed and gram positive organisms increased when the diet was changed from hay to grain causing a lowering of pH.

Golomzik and Ivanov (41) adapted cultures of Thiobacillus ferro-oxidans to increased iron and H^+ concentra-

tions by successive re-inoculations into liquid media with gradually increasing concentrations of these ions.

Pirt and Callow (42) studied the influence of pH on the morphology of Penicillium chrysogenum in submerged cultures. During steady state growth at a pH approximately 7, aberrant morphology was noted and pellicle formation was dependent upon pH. At pH 6, a normal filamentous growth was obtained. These investigators suggested that the resistance of the cell walls of the hyphae decreased with an increase in pH from 6.0 to 7.4.

Brookes and Sikyta (43) conducted studies using continuous cultures of Neisseria gonorrhoeae at pH values varying from 6.4 to 7.2. At pH values above or below 6.8, there was a marked decrease in cell concentration when the dilution rate fell outside the range 0.20-0.25 per hour.

Cseri (44) studied the effect of pH on some of the wood-rotting fungi, e.g., Stereum purpureum, Trametes versicolor, and Phellinus igniarius; the optimum pH for growth in soy-extract glucose medium varied with the fungal species. With the lowering of pH, the cell concentration of Trametes versicolor and Phellinus igniarius decreased gradually.

The chemical composition of cells has been observed to be dependent on the extracellular pH of the medium in which they are grown, as evidenced by the citations from the literature included below. Conner, Goldberg, and Kornacker (45) observed that accumulation of orthophosphate by

Tetrahymena pyriformis varied with the pH of the medium in which the cells were grown, the maximum concentration of orthophosphate occurring at a pH of 6.5. They reasoned that the mechanism concerned with orthophosphate entry into the cells involved specific sites in the cellular membrane which are sensitive to hydrogen ions.

Slyter, Bryant and Wolin (46) investigated the morphological and biochemical changes occurring in a continuous culture of rumen micro-organisms. Among other things, they found that the DNA concentrations (weight per unit volume of reactor liquid) decreased with decreasing pH. Also, protozoa were present in low concentrations during periods of acid pH values, a finding which is in agreement with that of Gibson (40). At pH 5, all strains of bacteria present were nonmotile and rod-shaped, and only 65 per cent were gram negative.

The uptake of metallic ions by microbial cells depends on the extracellular pH as shown by the following references. Conway and Beary (47) found that the uptake of Mg^{++} by yeast cells was dependent on pH and the strongest inhibitor of Mg^{++} uptake was H^{+} ion.

Armstrong and Rothstein (48) working on the "discrimination" in uptake between alkali metal ions by yeast, noticed that the pH of the medium markedly influenced the ability of yeast cells to discriminate between K^{+} and Na^{+}, K^{+} uptake being favored to a greater degree at low pH. They also studied the kinetics of uptake of these ions with

respect to pH. Between pH 6 and 8, H^+ had no effect; below pH 4, H^+ competitively inhibited the transport of each ion. Between pH 4 and 6, H^+ acted primarily as a noncompetitive inhibitor.

In addition to the foregoing effects, more than anything else, pH affects the enzymatic reactions of any living cell. Some observations made by research workers are given below.

According to Leitner and Cohen (49), the penicillinase activity of Staphylococcus aureus strain 55-c-1 increased as the pH of the medium dropped from 6.0 to 4.7. Their evidence indicated that the changes in enzymic activity were a function of the rate of formation of the enzyme.

The earlier findings of Gale and Epps (37), quoted elsewhere, described clearly the role of pH in determining the activity of amino acid deaminase and decarboxylase in bacteria.

The release of metabolic intermediates and/or end products is dependent upon the pH of the medium. Grechushkina (50) observed that the fermentation products of Lactobacterium pentoaceticum were highly dependent on pH. When pH was maintained at about 5.0, after 72 hours of fermentation, the product ratio lactic acid to alcohol was 3:2, whereas the ratio observed at pH 7.0 was 2:1. Under strongly acid conditions, the ratio at 46 hours of fermentation was 1:2. He argued that the predominant accumulation of alcohol in strongly acid conditions should be regarded as

the means by which the above bacterium reacted against high acidity of the medium. This is in agreement with the mechanism of alteration of pH by Escherichia coli discussed formerly by Gale and Epps (37).

Kozlova and Sapozhnikova (51) examined the growth of Pseudomonas fluorescens and Pseudomonas denitrificans. The growth of these species on sugars was accompanied by the production of acids such as lactic, gluconic, and succinic, and these formations were highly pH-dependent. In the case of Pseudomonas fluorescens, according to these investigators, glucose consumption resulted in the formation of lactic acid and accumulation of bacterial cells.

Nakata (52) grew cultures of Bacillus cereus strain T in buffered and unbuffered glucose-yeast extract-mineral salts medium. He found that acetic acid and lactic acid were the chief end products of growth in buffered media. More than twice the amount of these acids accumulated in the buffered medium than was found in the unbuffered medium. In the latter cultures, pyruvic acid rather than lactic acid was found together with acetate. Some acetoin was also found. He also noticed that all of these intermediates or end products were rapidly utilized once sporulation began. Poly-β-hydroxy butyric acid accumulation in the cells during initial stages of sporulation was seen to be pH-dependent.

There is very little information in the literature regarding the effect of changes in environmental pH upon

endogenous respiration. Dawes and Ribbons (53) stated in their review article that alteration of environmental pH did not affect the endogenous respiration of Pseudomonas aeruginosa, Bacillus prodigiosus, Bacillus proteus, and Euglena gracilis.

Having presented a brief review of studies made using pure cultures, it seems pertinent to examine investigations made on the effects of pH in the area of waste water treatment.

Baly (54), as early as 1931 considered the part played by pH in the treatment of sewage by the activated sludge process. He discussed the electrical charges on sewage colloids with respect to the isoelectric point (pH approximately 6.5) of sewage, but failed to describe the effects of pH on removal of dissolved matter in sewage.

Ingols and Heukelekian (55) enumerated the compounds causing buffering action in sewage. They attributed the buffering action in sewage to the presence of ammonium bicarbonate, fatty acids (soaps), phosphate, humic acids, uric acid, amino acids, peptones, and peptides. The reasons for loss of buffer effects in activated sludge were listed as (1) loss of carbon dioxide by aeration, (2) oxidation of organic acids, (3) conversion of ammonia to nitrates, (4) production of more free carbon dioxide with concomitant removal of ammonium ions.

Ruchhoft, Kachmar, and Moore (56) investigated the oxidation of glucose by activated sludge at pH values from

3.9 to 11.5 with periods of aeration varying from 30 minutes to 22.5 hours. Glucose removal was practically nil outside the pH range 3.9-9.6. In a study of the growth requirements of Sphaerotilus natans, Ruchhoft and Kachmar (57) observed that this filamentous organism was more sensitive to pH than activated sludge itself, and its optimum pH range was 6.0-9.0.

Butterfield (58) studied the pH requirements of a "zooglea-forming" bacterium isolated in pure culture from activated sludge. Even though it grew over a pH range of 5.6-8.5, the optimum seemed to be 7.0-7.4.

Woolridge and Standfast (59) examined the oxygen uptake of sewage-activated sludge mixtures at pH values ranging from 4.0-10.0 using Barcroft differential manometers. They found that most rapid oxidation occurred between pH 6.0 and 9.0, with the optimum at 7.3.

Levine and Soppeland (60) studied the proteolytic activity of different organisms isolated from activated sludge, using gelatin and milk proteins as carbon sources. They observed that proteolysis was optimum in the pH range 7.0-7.5.

Keefer and Meisel (61) conducted some studies on the effect of pH on the activated sludge process. The best performance, as judged by "oxygen consumed test," was at pH 7.0-7.5. Purification efficiency was almost as good at pH values 6.0-9.0, which was in agreement with the findings of Woolridge and Standfast (59). Keefer and Meisel also

noticed that the raising or lowering of the pH on either side of neutrality greatly reduced the sludge-settling index.

Changes in pH cause microbial predominance changes in activated sludge. Cooke, Moore, and Kabler (62) studied BOD exerted by some fungi, and observed that

> "Oxygen-depleting capacity of fungi used in this work equal or exceed those of B. aerogenes and other bacteria in pure culture at pH 7.2. Fungus activity in pure culture increased as the reaction of the medium became more acid, and decreased as it became more basic."

Molds in general were more tolerant to low pH. The strains of fungi tested (Fusarium, Geotrichum, and Penicillium) were able to compete successfully with other micro-organisms for dissolved organic matter and oxygen.

Rogers and Wilson (63) observed that reduction in microbial population of sewage and river water occurred rapidly in the pH range 4.5-3.5. In acid pH, the predominating species were mostly gram negative organisms and yeasts.

Jones (64) in his study of biodegradability noticed that the growth rate of bacteria at pH 7.0 was five to ten times higher than that of fungi at the same pH; while at pH 2.0 the bacteria either died off or formed spores, whereas fungi could still grow. This result indicates that severe shifts in pH could cause severe changes in microbial predominance in mixed systems.

Treatment of industrial wastes has been practised over

wide ranges of pH. Some observations from the literature are given below. Heukelekian and Gellman (65) studied biochemical oxidation of various trade effluents at different pH values. In board mill whitewater, the oxygen uptake rate was not affected within a range of pH values from 6-8. At pH 5 and 9, the oxygen uptake was retarded. The oxygen uptake was satisfactory in the pH range 6-9 for rope cook liquor. Antibiotic wastes were purified better at acid than at alkaline pH values. Spent broth could be oxidized successfully in the pH range 5-9; the same was true of slaughterhouse waste.

Atkins and Sproul (66) have stated that potato waste could be treated by a complete mixing activated sludge system without pH adjustment of the influent. The biological solids level in the aerator was held at 4000 mg/l, and the detention time employed was 6-8 hours.

From the foregoing review on the studies of pH, it is seen that no systematic effort has been made to delineate the biochemical changes incidental to pH shock loads in a complete mixing system.

D. Effect of Temperature on Microorganisms and Continuous Cultures

Of all the physical agents that influence the life and activities of micro-organisms, temperature stands out as a prime factor due to its control over the rate of chemical reactions. No other aspect has received more attention in the study of the biochemistry of micro-organisms.

The effect of temperature on intracellular matter has been studied by Evreinova, et al. (67). They found that an increase of the growth temperature of the fungus Aspergillus fumigatus from 25 to 50°C was accompanied by a decrease in the amount of nucleic acids and by a change in the nucleotide composition of RNA. However, the composition of DNA remained constant.

Evreinova, et al. (68) investigated the intracellular effects of temperature on thermophilic and mesophilic strains of Saccharomyces cerevisiae. They found that an increase in temperature was accompanied by reductions of 20.5 per cent in the nucleic acid content, 25 per cent in the protein content, and 43 per cent in cell weight.

Marr and Ingraham (69) found that there was no direct relationship between fatty acid composition and minimal growth temperature in Escherichia coli.

Evreinova, et al. (70) observed that in the mycelium of Micromonospora vulgaris cultivated at 56°C, there was a decrease in the total content of nucleic acids and some diminution in the amount of the guanine-cytosine pairing as compared with the mycelium grown at 40°C. With a decrease in temperature from 56 to 40°C, the RNA level increased and a slight change was noted in the nucleotide composition toward an increase of the guanine-cytosine pair. Mycelium grown at 56°C contained 55 per cent protein and at 40°C, 59 per cent.

The effect of temperature on the cell wall composition

of some thermophilic bacilli was investigated by Forrester and Wicken (71). They grew Bacillus stearothermophilus and Bacillus coagulans in a glucose-salt medium containing trypticase-yeast extract at 37°C and 55°C. The higher temperature caused an increase in mucopeptide and decrease in teichoic acid of the cell walls of both organisms. They also found that the lipid contentwas higher than the usual value (1-2 per cent) in mesophilic gram positive bacteria.

The growth temperature has been found to affect the morphological characteristics of several micro-organisms. Terry, et al. (72) found that the vegetative cells of Clostridium acidiurici were straight rods 2.5 to 4.0 microns in length, when grown at temperatures up to 42°C. When grown at 43°C, the cells showed a definite tendency to elongate, and at 44°C, filaments often exceeding 500 microns in length were formed. Only an occasional cross wall was apparent in the heat-induced long forms, but as the temperature was lowered, they formed cross walls readily and fragmented into single short cells. The filaments grown at 44°C were gram negative, whereas cells grown at 37°C were gram positive. They suggested that the rapid transition from filaments to single cells upon lowering the temperature from 44 to 37°C was a phenotypic response rather than a mutation.

Changes in temperature are known to alter growth requirements of several micro-organisms. These requirements become progressively numerous with increased or decreased

temperatures. The data in Table I taken from Langridge (73) provide an interesting insight into this phenomenon.

TABLE I

NUTRIENT REQUIREMENTS AT HIGH AND LOW TEMPERATURES

Organism	Strain	Temperature Differential °C*	Requirement, Stimulant, or Blocked Reaction
A. Nutrient Requirements at High Temperatures			
Bacillus coagulans	32	45/36	desthiobiotin to biotin
Bacillus megaterium	lysogenic	55/37	yeast extract, beef extract, and proteose peptone
Bacillus stearothermophilus	5274	55/45	pimelic acid to desthiobiotin
Bacillus subtilis	2 strains	55/37	yeast extract, beef extract, and proteose peptone
Escherichia coli	-	44/37	glutamic acid and nicotinamide
Escherichia coli	-	37/24	methionine or p-aminobenzoic acid
Proteus vulgaris	-	40/25	nicotinamide and thiamine or one of 12 amino acids
Saccaromyces cerevisiae	2 strains	38/30	pantothenic acid
B. Nutrient Requirements at Low Temperatures			
Bacillus coagulans	12	36/45	valine
Bacillus stearothermophilus	6 strains	37/55	tryptose and basamine

*The first temperature is that at which the temperature requirement is manifest; the second is that at which the requirement is not manifest.

Grechushkina (74) observed that incubation temperatures lower than the optimum (37°C) could alter the metabolism of the heterofermentative lactic acid bacteria Lactobacterium pentaoaceticum. Under optimal conditions of acidity and aeration, at 43 and 48-50°C, the division of bacteria was inhibited, the amount of lactic acid production increased, and alcohol decreased. At 48-50°C, it became homofermentative. At 23 and 30°C, cell division was stimulated, lactic acid decreased, and alcohol increased.

One of the recent findings, which has not been properly explained, is the interaction between structure and viscosity of water and biological activity. Water in the liquid phase has been shown to undergo changes in structure and viscosity at temperatures of 15, 30, 45, and 60°C. These points of discontinuity have been shown to affect physiological processes in the growth of micro-organisms. Davey, et al. (75) studied four bacteria, Pseudomonas fragi, Streptococcus faecalis, Bacillus coagulans, and Bacillus stearothermophilus strain 1518-smooth, at temperatures of 5-20°C, 20-40°C, 35-55°C, and 50-70°C, respectively. In all cases, growth was suppressed at or near the temperatures which caused a change in the structure and viscosity of water, suggesting a strong interaction between the structure and viscosity of water and biological activity.

The effect of a rapid change in temperature on different types of cultures deserves further consideration, since it is related to the studies undertaken in the present

research. Gorril and McNeil (76) noticed that young actively dividing cultures of Pseudomonas pyocyanea at 37°C were killed when diluted into cold liquids at less than 18°C. The cold shock was more effective on young than on old cultures, and when the diluent was distilled water. Under similar conditions, Staphylococcus aureus was resistant to cold shock.

Ng, et al. (77) studied the effect of transient temperatures on growth rate. They subjected exponentially growing cultures of Escherichia coli ML30 to rapid changes of temperature. If the change in temperature was made within the range of temperatures in which the temperature characteristic* μ was a constant, exponential growth resumed immediately. But if the shifts were made to or from a temperature below the range of constant μ, the initial growth rate was intermediate between the rates normal for the initial and final temperatures. They reasoned that growth at low temperature altered or damaged the cell in a way that reduced the growth rate. They also found that glucose could not repress the induction of β-galactosidase at these low temperatures, and therefore de-repression might be the damage that resulted from growth at these low temperatures.

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*$\log k = \frac{\mu}{2.303\ RT} + C$, where
k = specific growth rate
μ = temperature characteristic
R = gas constant
T = absolute temperature
C = constant

Strange and Dark (78) found that the lethal effect of cold shock on Aerobacter aerogenes suspensions depended on the time of exposure to low temperatures, the growth phase, the concentration of the bacteria, and the diluent. They found that the presence of certain substances like amino acids, adenosine triphosphate (ATP), nucleic acid constituents, sucrose, and Mg^{++} in the cold diluent afforded protection against lethal effects.

Strange and Shon (79) investigated the effects of thermal stress on the viability and RNA content of Aerobacter aerogenes. They found that the death rate of washed aqueous suspensions of the organism at 47°C depended on the nature of the growth medium, the composition of the liquid used to wash and re-suspend the bacteria, the bacterial growth phase, the bacterial concentrations in the heated suspensions, the pH value, the oxygen tension, and the composition of the diluent in which the bacteria were heated. They also found that starvation increased the thermal resistance. The above conditions which accelerated the death rate of bacteria also increased the rate of degradation of endogenous RNA.

Using yeasts, Shaw (80) has investigated and confirmed the general findings pertaining to growth rates in relation to the temperature characteristic described previously by Ng, et al. (77), who studied Escherichia coli.

Having made a review of recent developments pertaining to temperature effects for various pure cultures, it is

expedient to note the developments which have been reported in the waste treatment field. Sawyer and Nichols (81) observed that oxygen utilization by activated sludge was dependent on temperature, and previous acclimation to a selected temperature had no effect on the relationship. Sawyer (82) conducted a series of laboratory experiments to determine the effect of temperature and the concentration of biological solids on the efficiency of activated sludge. He obtained almost identical BOD removals under all conditions, except at low solids levels and at a temperature of 10°C. In contrast with Sawyer's conclusions, Bloodgood (83) stated that "temperature of the sewage definitely plays an important part in determining the capacity of an activated sludge plant." His investigations included plant studies at Indianapolis, Indiana, from 1935 to 1939.

Ruchhoft and Kachmar (57) studied the effect of temperature on the growth of Sphaerotilus and found that the organism exhibited a narrower range of optimum temperature than activated sludge.

Gotaas (84) concluded that at 30°C and above, there may be considerable nitrification taking place during the apparent first stage of BOD exertion. He also noticed that the lag period increased with low temperatures.

Gehm (85) reported that at a detention time of 4 hours and solids concentration of 3000 mg/l, activated sludge pilot plants could be operated successfully at temperatures as high as 52°C, and no difficulty was experienced in main-

taining aerobic conditions.

Ludzack, et al. (86) conducted an exhaustive study of the effects of temperature on the performance of activated sludge. The activity of the predators at 30°C (rotifers, nematodes, stalked ciliates) was higher than at 5°C (flagellates, stalked cilites, and free-swimming ciliates). They found that an acclimation period of two weeks was required when a change in temperature was effected. Solids production was higher at low temperature. At low temperature, the nitrogen requirements were lower and sludge was not easily flocculated. Their observation that Sphaerotilus had a better competitive position at 5°C is in apparent disagreement with that of Ruchhoft and Kachmar (57), who stated that the organism was more sensitive than activated sludge to low temperatures.

A few of the studies on the effect of temperature on the treatment of industrial wastes is given below. Amberg and Rudolphs (87) have concluded that successful treatment of whitewater and whitewater concentrates (board-mill wastes) is possible in both the mesophilic (30-40°C) and thermophilic (50°C) range of temperature.

Dougherty and McNary (88) conducted studies on the effects of elevated temperatures on the aeration of citrus wastes. Temperatures as high as 36°C did not have any damaging effects on the system. A rapid rise in temperature of 4 or 5°C caused temporary disruption, but the system recovered in due course provided the temperature did

not change any further. An increase from 36 to 43°C caused reduction in the number and activity of protozoa. At 46°C, purification efficiency was retarded and the experiment was terminated.

Hurwitz, et al. (89) studied the degradation of cellulose by activated sludge. They concluded that temperature exerted a definitive influence. At 12-13°C, only 6.7 per cent of the cellulose was degraded in 72 hours, whereas at 23°C, 87 per cent was degraded. According to this report, degradation of cellulose appears to be highly temperature-dependent.

From the foregoing review of literature in the fields of microbiology and pollution control engineering it is noted that studies have not been undertaken in any great depth, which shed light on the biochemical changes consequent to the imposition of transient conditions which involve a change in dilution rate, pH, or temperature. It is felt that the advisability of employing combined treatment of sewage and industrial wastes, or of various combinations of industrial wastes can be decided upon more intelligently in any individual case if the basic biochemical events consequent to the changes brought about by this practice are delineated. The delineation of these effects can be accomplished in the laboratory using bench scale reactors and analytical techniques usually employed in the fields of biochemistry and microbiology as well as those commonly employed in the pollution control field. It is to

this aim that this research pertaining to the effects of changes in flow rate, pH, and temperature herein reported were conducted.

CHAPTER III

THEORETICAL CONSIDERATIONS

A. Theory of Shock Loads

The functional design of activated sludge systems has been based primarily on data collected from sludges acclimated to a single waste and to various conditions of operation, i.e., pH, temperature, and flow rate (detention time). However, sometimes for economic reasons, different wastes may have to be treated in the same reactor, and this will require accumulation of data regarding transient as well as steady state parameters, especially when the waste streams are not coincident with respect to their peak flows and physical and chemical characteristics. When treatment plants were designed primarily for purification of domestic wastes, the major type of "shock load" that was envisaged was an increase in the organic or BOD loading. However, there are several different types of waste stream changes which can be classified as shock loads. In a broad sense, any rapid or abrupt change in the physical or chemical environment in a biological system may be classified as a system shock or shock load.

The major types of shock loads which may impair plant

efficiency are described below:

1. Quantitative shock load: This type of shock loading involves generally an increase (or sometimes decrease) in the concentration of the biologically degradable organic matter or BOD in the influent. This type of shock load occurs in all treatment plants whether or not they treat one or multiple types of wastes.

2. Qualitative shock load: This type of shock loading involves a change in the chemical structure of the substrate, i.e., a change in the structural configuration of the carbon source. This concept of a shock load was originally described by Gaudy (18). He theorized that a successful response to such a shock load depended upon changes in predominance, shifts to different metabolic pathways, and the induction of necessary enzymes.

3. Hydraulic shock load: This involves a change in the rate of flow of the influent waste stream which causes a change in detention time in the aerator. This type of shock load may or may not be accompanied by a concurrent change of organic matter in the influent. Thus a hydraulic shock load may frequently be accompanied by a quantitative shock load and the system response may be adversely affected. The occurrence of a hydraulic shock load is incidental to variations in waste flow caused by hourly variations of water usage, both domestic and industrial, and is of considerable importance where combined sewers are in use.

4. pH shock load: As the name implies, this type of shock load involves a change in the pH of the incoming waste. In many cases these changes may be the result of admission of industrial wastes which are acidic or alkaline in nature. This type of shock load may be more important from a biochemical point of view than all the shock loads discussed thus far, because almost all micro-organisms and enzymatic reactions are highly pH-dependent.

5. Temperature shock load: This type of shock load may be caused by a rapid change in the temperature of the influent waste stream or reactor. Admission of cooling waters from industries may result in such a condition. As with pH, this is another type of shock load which can adversely affect the metabolic activities of micro-organisms in the reactor.

6. Toxic shock load: This type of shock loading involves an influx of wastes which contain certain toxic components; e.g., heavy metals which disrupt the established metabolic reactions in the activated sludge.

B. Response of Activated Sludge to Shock Loads

In general, response to any shock load will depend upon the new environment resulting from the change as well as the immediate past history of the system. If the change results in an adverse environment, the biochemical activities of the activated sludge may be decreased. Also, the flocculation and settling characteristics of the sludge may be affected. A successful response will depend upon several

factors which may include the following:

1. The severity and/or the rapidity of the shock load.

2. The detention time in the reactor.

3. The physiological characteristics of the sludge, e.g., sludge age.

4. The sludge concentration in the aerator.

5. The oxygen tension in the aerator.

6. The predominating microbial populations in the sludge and the number of different species present, i.e., the degree of heterogeneity.

Completely mixed activated sludge systems may be defined simply as continuous cultures of mixed populations. Excluding the situation caused by transient conditions, they operate in a dynamic steady state, continuously converting the reactants (organic matter) into products (cells and metabolic products), but never reaching equilibrium. Upon the application of a transient, i.e., one or more of the previously enumerated shock loads, several control mechanisms are set in motion, so that the population adjusts to the environment. Even though the studies conducted in this research are concerned more with the effects of shock loads on the sludge and substrate removal efficiency, than with the basic control mechanisms (cellular), a discussion of the mechanistic aspects is of considerable value since this information may help in analysis of the observed responses.

From a mechanistic point of view, the response of mixed

populations to an environmental change may be classified as either intracellular or intercellular. The former includes changes in the types, concentration and activity of the enzymes, the rate of chemical reactions, and the biochemical components in the cells, whereas the latter involves a competitive effort between the different species present in the biological population making up the activated sludge. Stated in another way, the intracellular response involves biochemical acclimation of all or a portion of the population, whereas the intercellular response involves adaptation of the population, resulting in a natural selection of the organisms best suited for the new environment.

Intracellular response may be the resultant of two separate responses, i.e., a response controlled by enzyme synthesis or repression of synthesis , and a response due to an effect on the activity level of the enzymes existing in the system. Both modes of response can lead to changes in the efficiency of the micro-organisms and in the case of a successful response, can permit more rapid growth. Bacteria growing at different rates have different levels of intracellular constituents, i.e., protein, carbohydrates, lipids, RNA, and DNA. Since hydraulic dilution rate controls bacterial growth rate in a completely mixed continuous flow reactor, the application of a hydraulic shock load tends to induce a parallel change in the growth rate of micro-organisms. However, the organisms may require a period of adjustment before they reach the new steady state

growth rate. This may be accompanied by parallel changes in the intracellular constituents, e.g., RNA.

Perret (36) in his discussion of a new kinetic model for growing bacteria, has postulated the existence of a phenomenon he termed "growth rate hysteresis" with respect to transient conditions of nutrient concentration. According to his theory, the specific growth rate at an increasing transient of nutrient concentration will be lower than the corresponding value for steady state conditions. Similarly, for decreasing nutrient concentration, the value of the growth rate will be higher than that produced by the existing substrate concentration, Even though he did not consider the effects of a change in dilution rate, it is possible that a similar phenomenon may occur during hydraulic shock loads which cause an increase or decrease in the dilution rate.

According to Hewitt (90), the response of a bacterial cell to changes in environmental pH may be "compensatory, adaptive, or permanent." In compensatory changes, the behavior of the cell returns to the original pattern immediately the pH is changed back to its former value. The compensatory mechanism may be operative for very small changes in pH of the medium, and brings about an increase in the level of enzymes which release into the medium an intermediate or end product tending to bring the pH back to its original value. This mechanism has been described in detail by Gale and Epps (37). The second mechanism of response of bacteria exposed to a change in pH of the environment may be

the formation of adaptive or inducible enzymes which may persist in the cell for a time after the original conditions are restored. Mechanisms involving adaptation of enzymes have been discussed fully in "Adaptation in Micro-organisms" (91). Severe changes in pH may result in death of the bacterial cell.

In the previous chapter it was mentioned that a change in temperature could cause a requirement for a specific substance in order to stimulate growth. According to Langridge (73), the possible causes for high temperature growth requirements include:

1. Availability of gases (e.g., carbon dioxide, oxygen): Carbon dioxide in solution is required for growth of many micro-organisms. For example, Borek and Waelsch (92) have shown that in Lactobacillus arabinosus, phenylaline and tyrosine were necessary for growth at 37°C but not at 26°C, and that aspartic acid became an essential nutrient at 39°C. They found that if CO_2 tension was increased, these amino acids were not required at higher temperatures. They argued that CO_2 may be involved in the synthesis of these amino acids. Now it is known that CO_2 is required in the conversion of pyruvate to oxalo-acetate which is involved in the synthesis of aspartate.

The solubility of oxygen will also be affected, and it is possible that at high temperatures oxygen tension may limit microbial activity.

2. Accelerated breakdown of cell components: It is

possible that at elevated temperatures the breakdown of intracellular matter may be faster than its synthesis.

3. Occurrence of rate imbalance including a decrease in the coordination of interrelated syntheses, the onset of various types of inhibition, and an increase in the velocity of destructive as opposed to synthetic reactions.

4. Nonformation of inducible enzymes.

5. Reversible and irreversible heat inactivation of enzymes.

In his review article, Langridge has substantiated these theories by citing examples from the literature.

The effect of low temperatures may involve a consideration of activation energies and hydrogen bonding of the enzyme systems (93). However, these theories have not been substantiated by adequate proof and do not warrant discussion.

The above theories on intracellular response are not intended to be tested with the results of these investigations on shock loads. However, they were presented so that they may be helpful in the discussion of the biochemical changes which occur as a result of shock loading.

The intercellular mechanisms cause ecological changes in heterogeneous systems such as activated sludge, and may be the cause for changes in predominating species during hydraulic, pH, or temperature shock loading conditions. According to Brock (94), it is possible to distinguish three main groups of organisms in an ecosystem.

1. Dominants, i.e., those possessing the greatest activity in the system, and thus having a controlling influence on the grouping of other organisms.

2. Associates, i.e., those which are dependent for their development on the activities of group 1, and

3. Incidentals, i.e, those which are more or less indifferent to the actions of groups 1 and 2 and which in turn have no influence on others.

In general, in a highly non-selective environment, e.g., municipal sewage containing many carbon sources, numerous species may be expected to grow and no one specie will attain a high degree of predominance. Also, it may be expected that an equilibrium between a large number of species would exist. However, shock loading conditions may impose a selective pressure on the environment in which many species will not be able to grow, and the most successful competitor(s) will predominate. In addition, shock loading conditions may result in the formation of new compounds, i.e., metabolic intermediates and/or end products produced by one group of organisms which may become the substrate for another group, and thus may affect the relative predominance of each group. In the experimental research accomplished for this dissertation, an attempt was made to observe gross changes in microbial predominance in response to shock loads admitted to each system, but no attempt was made to identify the species.

CHAPTER IV

MATERIALS AND METHODS

A. Experimental Apparatus

The experimental apparatus (pilot reactor) used in the studies is shown in Figure 1. The aeration vessel was made of Pyrex glass (net detention volume 2.4 liters). Air was supplied through fritted glass diffusers at a rate of 5000 cc/min. The reactors were maintained at a temperature of 25 $\pm$ 0.5°C except during temperature shock loading experiments. Temperature control was provided by a Precision "Lo/Temptrol" unit which was connected to the water bath in which the reactors were suspended. The detention time was controlled by varying the rate of inflow of the synthetic waste. The pump and motor unit employed for pumping the waste was manufactured by Milton Roy Company (Model 4-c-48-R). Suction and delivery lines were made of tygon tubing with glass junctions. Except for experiments in which recirculation of biological solids was employed, the mixed liquor was wasted direct from the aerator. The settling cones made of Pyrex glass were used to collect the sludge for recirculation to the aerator. Recycle sludge was obtained by drawing off settled sludge and diluting it to the desired concentration. Sludge for recirculation was

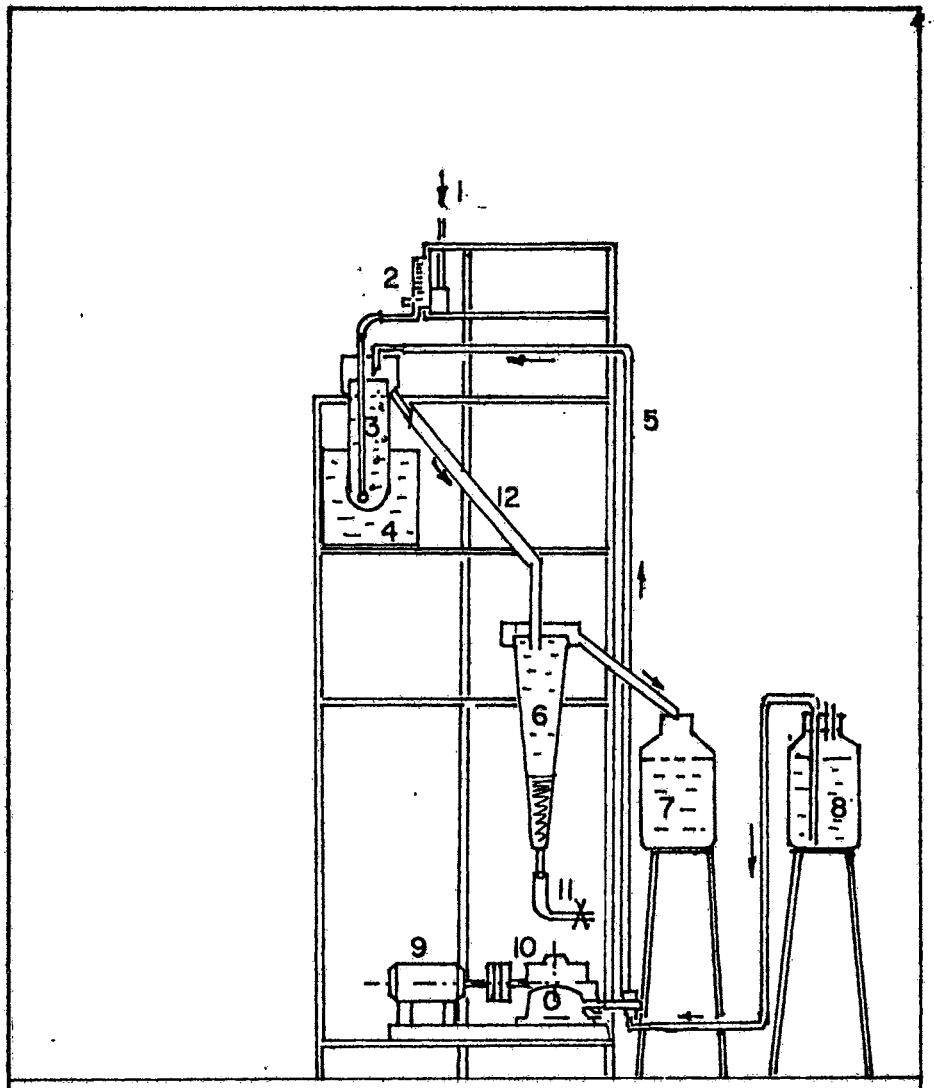

Figure 1 - Schematic representation of the experimental unit.

drawn off from the settling tank every twelve hours and was placed in a batch aeration vessel from which it was pumped back to the aerator. A Sigmamotor pump (Model T8) was used for pumping the sludge back to the aeration vessel.

The reactor was tested for complete mixing by filling it with distilled water, and then pumping in a solution of methyl red of known concentration and measuring the methyl red concentration of the effluent at various time intervals. A plot of the concentration versus time followed the theoretical expression $x = x_o(1-e^{-Dt})$ where

x = concentration in the reactor or effluent at any time t

x_o = concentration in the influent, and

D = dilution rate,

thus ensuring the requirement of complete mixing in the reactor.

B. Composition of Synthetic Wastes

Synthetic waste employing carbohydrate (glucose) as the carbon source was used in all experiments. The composition of the waste (except under pH shock loading conditions, for which the phosphate compounds were varied) was as follows: glucose - 1000 mg/l, $(NH_4)_2SO_4$ - 500 mg/l, $MgSO_4 \cdot 7H_2O$ - 100 mg/l, $CaCl_2 \cdot 2H_2O$ - 7.5 mg/l, $MnSO_4 \cdot H_2O$ - 10 mg/l, $FeCl_3 \cdot 6H_2O$ - 0.5 mg/l, KH_2PO_4 - 527 mg/l, K_2HPO_4 - 1070 mg/l, tap water - 100 ml/l, and distilled water to volume. This waste was designed to make the carbon source the limiting nutrient.

C. Development of the System in Steady State Prior to Administering the Shock Loads

Each experiment was initiated by seeding the synthetic waste (2 liters) with sewage (20 ml) from the primary clarifier of the sewage treatment plant, Stillwater, Oklahoma. The system was aerated under batch conditions for a period of 24-48 hours to develop sufficient microbial growth. The system was then fed with synthetic waste by continuous pumping from a feed reservoir. The feeding was continued for a period of 3-5 days, during which steady state conditions were established prior to the initiation of any shock loading. Steady state conditions were checked by periodic measurement of the optical density of mixed liquor samples from the reactor, using a spectrophotometer. In recirculation experiments the period for development of steady state varied from 2-3 weeks.

D. Shock Loading Procedures

After having ensured the development of steady state conditions in the reactor, shock loading procedures were initiated.

1. Hydraulic Shock Loads

In the hydraulic shock loading experiments, two specific conditions were studied. In the first set of experiments the conditions studied were termed as shock loads with "constant organic concentration." In this type of experiment the glucose concentration in the feed was maintained constant (1000 mg/l) at all times, and the flow

rate (detention time) was varied either up or down, from that which would yield an eight-hour detention time. It can be seen that under conditions of constant concentration of carbon source in the inflow, the daily organic loading either increased or decreased in proportion to the increase or decrease in flow rate.

In other experiments termed "constant organic loading" experiments, an increase or decrease in flow rate was compensated for by a corresponding decrease or increase in the concentration of the glucose in the feed, so that the daily organic loading was constant. For example, if the flow rate was doubled, the glucose concentration in the influent was halved.

In this type of experiments, as well as for the "constant concentration" studies, the steady state flow rate maintained before applying any shock load was one which provided an eight-hour detention time in the aerator, i.e., the eight-hour detention time was used as a base value for flow rate. Selection of this detention time provided a base upon which the various shock loads could be compared. Any base flow could have been chosen, but the eight-hour detention time is one usually employed for the standard activated sludge process, and it seemed desirable to obtain large amounts of data for steady state at this detention time.

2. pH Shock Loads

In pH shock loading experiments, the ratio of the phos-

phorus compounds was varied, but a COD/P ratio of 3.4/1 was maintained at all times. The following table shows the amount of the phosphorus compounds used, and the pH values of the feed solution for the various experiments during shock loading conditions.

TABLE II

COMPOSITION OF PHOSPHATE BUFFERS FOR pH SHOCK LOADS

Phosphate compounds per 2 liter of buffer stock solution				pH of feed solution*
H_3PO_4 ml	KH_2PO_4 gm	K_2HPO_4 gm	K_3PO_4 gm	
130.80	19.18	-	-	2.75
16.36	240.20	-	-	3.65
1.84	268.40	-	-	5.20
-	267.60	5.43	-	5.60
-	234.80	47.60	-	6.60
-	181.20	116.20	-	6.95
-	142.40	163.80	-	6.85
-	-	340.80	8.88	8.00
-	-	110.40	289.00	9.35

*10 ml of buffer stock solution were used per liter of feed solution.

Values of pH higher than those given in the table resulted in precipitation of salts from the feed, and hence were not employed for shock loading studies. All studies on pH shock loading were conducted at a flow rate which

yielded an eight-hour detention time in the aerator. In recirculation experiments, the rate of sludge recycling was maintained at 33 1/3 per cent of the rate of flow of the synthetic waste into the aerator.

3. Temperature Shock Loads

The temperature shock loading experiments were conducted within a range of 8-57.5°C. However, all experiments had a base or steady state reactor temperature of 25°C prior to the initiation of the shock loading conditions. All experiments were run without sludge recycling, and were conducted at four-hour and eight-hour reactor detention times. The change in temperature during shock loading conditions was effected by changing the temperature of the water bath in which the reactors were suspended.

E. Experimental Protocol

During all experiments, periodic samples were collected both before and after the shock load was administered. The pH of the sample was measured using a "Beckman Zeromatic" pH meter, and the optical density was determined using a Bausch and Lomb Spectronic 20 spectrophotometer. The samples were centrifuged in a Servall Superspeed Angle Centrifuge (type SS-1) at a rotor speed of 12,000 rpm (18,400 relative centrifugal force) for a period of thirty minutes. The solids were filtered through a Millipore filter (HA, 0.45μ), and the filtrate was collected for refrigerated storage prior to analysis. Where the biological solids were to be determined, the tared membrane filter was dried

at 103°C for two hours, cooled in a desiccator for one hour, and weighed. For determination of intracellular matter by biochemical methods, the wet solids were scraped from the filter and re-suspended in a known volume of distilled water. The re-suspended solids and the filtrate were preserved in a freezer prior to analysis.

The dissolved oxygen in the mixed liquor of the aerator was measured periodically, using either the Jarrel-Ash or a "Galvanic Cell (Precision Scientific) oxygen analyzer. This was done to determine whether the system was subjected to oxygen deficiency.

F. Analytical Techniques

The COD of the filtrate was determined according to Standard Methods (95). The carbohydrate content of the filtrate and the sludge and the protein content of the sludge were measured by the procedures outlined by Gaudy (96).

The procedures for RNA and DNA were as follows: An aliquot of the re-suspended solids was extracted with five per cent trichloracetic acid for fifteen minutes at 25°C. This was done to deplete the cellular pools, and remove other soluble materials. After centrifugation, the pellet was extracted for thirty minutes with 2:1 chloroform-methanol mixture at 25°C. Nucleic acids were removed from the residue by boiling for thirty minutes (100°C) with five per cent trichloracetic acid.

DNA was determined by the diphenylamine reaction

described by Burton (97). An aliquot of the hot trichloracetic acid-soluble material was diluted with an equal volume of 1 N perchloric acid, and made up to 2 ml volume with distilled water. Four ml of diphenylamine reagent were added. This reagent consisted of 1.5 gm of diphenylamine dissolved in 100 ml of glacial acetic acid containing 2 ml of concentrated sulphuric acid and 0.5 ml of a solution containing 16 mg of acetaldehyde per ml. The mixture was incubated for twenty hours at 30°C. Absorbancy was measured at 600mμ using a Bausch and Lomb Spectronic 20 spectrophotometer. The DNA standard was prepared by dissolving 0.4 mg of commercially-prepared calf thymus DNA in 1 ml of 0.005 M NaOH. Before use, this solution was diluted with an equal volume of 1 N perchloric acid and heated at 70°C for fifteen minutes.

RNA was determined by the orcinol method described by Morse and Carter (98). An aliquot of the hot trichloracetic acid-soluble material was made to 2 ml with distilled water. Five ml of a solution containing 0.1 gm of anhydrous ferric chloride in 500 ml of concentrated hydrochloric acid were added. This was followed by 0.3 ml of a solution containing 2 gm of orcinol in 20 ml of 95 per cent ethanol. After mixing, the tubes were heated at 100°C for twenty minutes. Volumes were adjusted to 7.3 ml with distilled water. Absorbancy was measured at 600mμ using a Bausch and Lomb Spectronic 20 spectrophotometer. The standard solution consisted of 400 μg of RNA dissolved in 1 ml of five per

cent trichloracetic acid.

Elemental analysis of sludge was made using a Model 180 "Carbon Hydrogen Nitrogen Analyzer" (Hewlett-Packard Company, Avondale, Pa.). The analysis is based on the conversion of the elements carbon, nitrogen, and hydrogen in the organic matter to gaseous end products (CO_2, N_2, H_2O) at temperatures of 700-1,000°C. The catalysts used in the oxidation process are Ag_2O, MnO_2, and cupric oxide. Nitrogen oxides, if found, will be reduced to nitrogen by reduced copper in the packing of the combustion tube. The products of combustion are confined in a closed loop chamber within the instrument until reaction is complete, and then the individual components are injected into a special dual column gas chromatograph employing a dual thermal conductivity detector. Four peaks result: (1) combined CO_2 and N_2, (2) H_2O, (3) N_2, and (4) CO_2. Only the last three are used in computations. The H_2O peak is related to the hydrogen, the N_2 peak to the nitrogen, and the CO_2 peak to the carbon content of the sample.

Volatile acids were analyzed by gas-liquid chromatography. A Model 810 "Research Chromatograph" (Hewlett-Packard Company, Avondale, Pa.) was used for this analysis. This instrument contains a gas-liquid partition column in which the separation of the components is achieved due to their differing partition coefficients between the stationary (high boiling liquid) phase and a mobile (inert carrier gas) phase. A small quantity of the sample mixture which

is to be separated is injected into the carrier gas stream through the injection port of the instrument. The injected sample is then immediately swept as a slug into the column. The individual components of the sample move through the column at different velocities, depending upon their partition coefficients between the liquid phase and the carrier gas.

A glass column with 3/16 inch internal diameter was used to contain the packing material. The column was packed by applying suction at one end of the glass column while admitting the packing material at the other end. Care was taken to see that no air was trapped along the length of the packed material in the column. After packing was completed, the ends were plugged with cotton to prevent loss of the packing material.

The packing material was "polypak 2," a polymer which is thermally stable up to 300^{o}C in an oxygen-free atmosphere. The properties of the polymer packing material are: surface area = 300 m^2/gm or 120 m^2/cc, density = 0.4 gm/cc, color = white, mesh size = 80/120. This material is capable of detecting air, carbon monoxide, carbon dioxide, water, ketones like acetone, methyl ethyl ketone, volatile fatty acids, aromatic and cyclic aliphatic hydrocarbons.

Helium was used as the carrier gas with a flow rate of 140 ml/min at 60 psi pressure. Hydrogen and air were supplied at 30 psi and 33 psi, respectively. Hydrogen flame was employed in the detector. The column temperature was

maintained at 190°C. The detector and injection port temperatures were maintained at 230 and 225°C respectively. Dual flame detection was adopted for the analysis. The sequential elution of the volatile acids is dependent on increasing numbers of carbon atoms. The peak area method was used to determine the concentration of the eluted acids.

CHAPTER V

RESULTS

The experimental data will be presented in three major sections dealing with (1) hydraulic, (2) pH, and (3) temperature shock loads. In general, the results of each experiment are presented in two figures, the first one giving such parameters as biological solids, filtrate COD, anthrone (carbohydrate) COD of the filtrate, cell protein, cell carbohydrate, pH, and temperature wherever each is available. In a second figure, immediately following, the percentages of protein, carbohydrate, RNA, DNA, etc., of the dry sludge are given. In all figures, the data to the left of the vertical dotted line indicate the prevailing steady state conditions prior to the initiation of the shock. The dotted vertical line indicates the time at which shock loading was initiated, and the data to the right of the dotted line show the post-shock conditions. The units along the abcissa are in hours with the origin at the point of shock. Negative values of time are used for pre-shock, and positive values for post-shock conditions.

A. Hydraulic Shock Loads

1. Constant Influent Organic Concentration Conditions

a. Decrease in Dilution Rate

Figure 2 shows the biochemical response of a system when its flow rate was reduced to 25 per cent of its steady state value. This caused a change in detention time from eight hours to thirty-two hours and in dilution rate from 0.125 $hour^{-1}$ to 0.031 $hour^{-1}$. It is seen that the biological solids level fluctuated widely as a result of the shock. There was improvement of the effluent COD; also the anthrone COD values were lower. On the whole, the system responded satisfactorily. Figure 3 shows the RNA and DNA content of the sludge. There was a small increase in the RNA content; the DNA content decreased to a very small extent. It was observed that the application of this shock load caused some changes in microbial predominance in the system as evidenced by changes in color and by microscopic observations.

Figure 4 shows a slightly milder shock; the dilution rate was changed from 0.125 $hour^{-1}$ to 0.062 $hour^{-1}$, which caused a change in detention time from eight to sixteen hours. Biological solids concentration was not affected for the first eighteen hours; however, after this period there was a small decrease in the solids level. There was some increase in the filtrate COD and anthrone COD. There was an increase in the total cell protein, shortly after the application of the shock, and this increase in protein was at the expense of cell carbohydrate. The sludge composition expressed as percentages is given in Figure 5. There was an

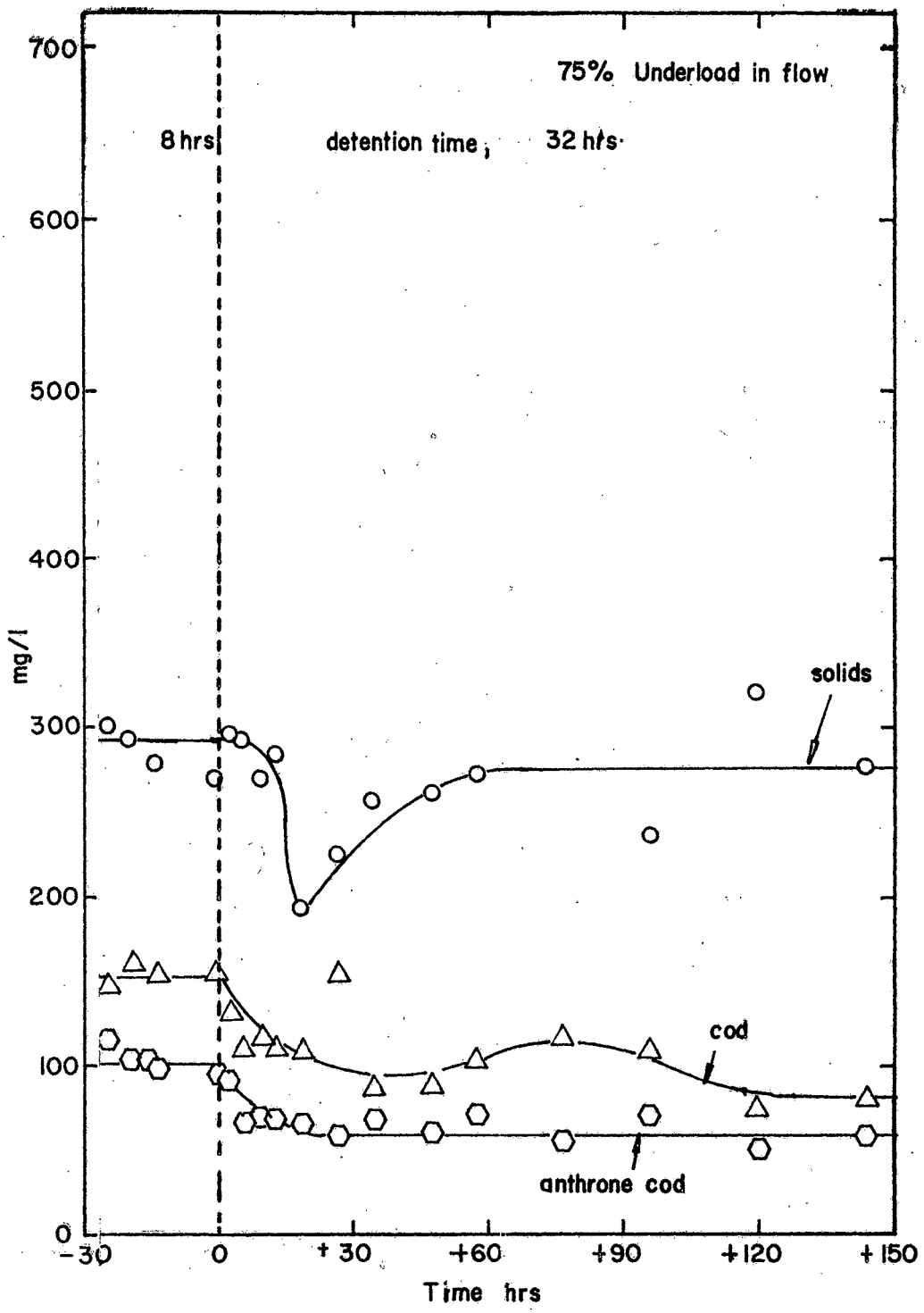

Figure 2 - Response of a steady state system shock loaded from a dilution rate of 0.125 hour^{-1} to a dilution rate of 0.031 hour^{-1} (constant concentration).

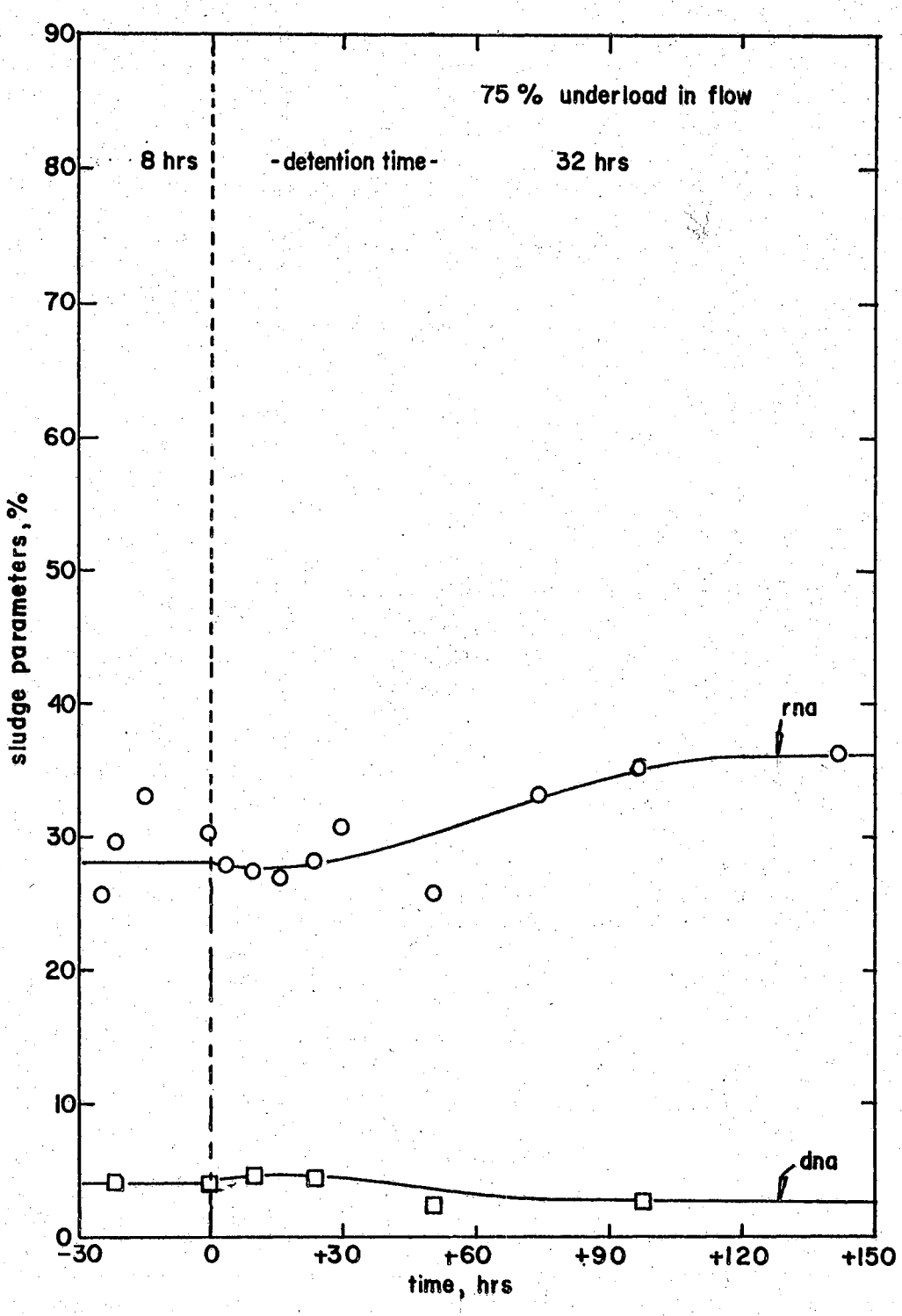

Figure 3 - Change in sludge composition of a system shock loaded from a dilution rate of 0.125 hour^{-1} to 0.031 hour^{-1} (constant concentration).

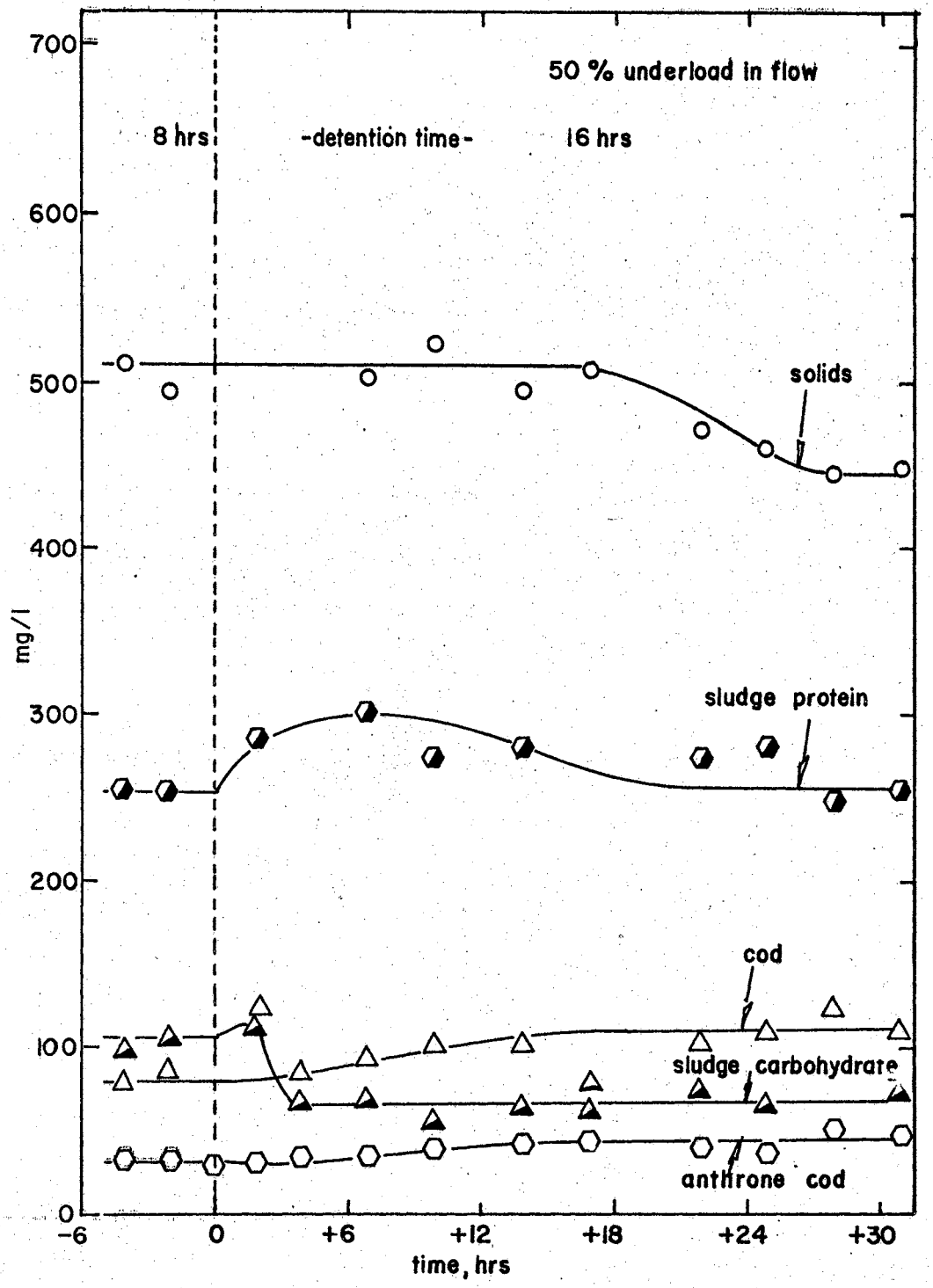

Figure 4 - Response of a steady state system shock loaded from a dilution rate of 0.125 hour^{-1} to 0.062 hour^{-1} (constant concentration).

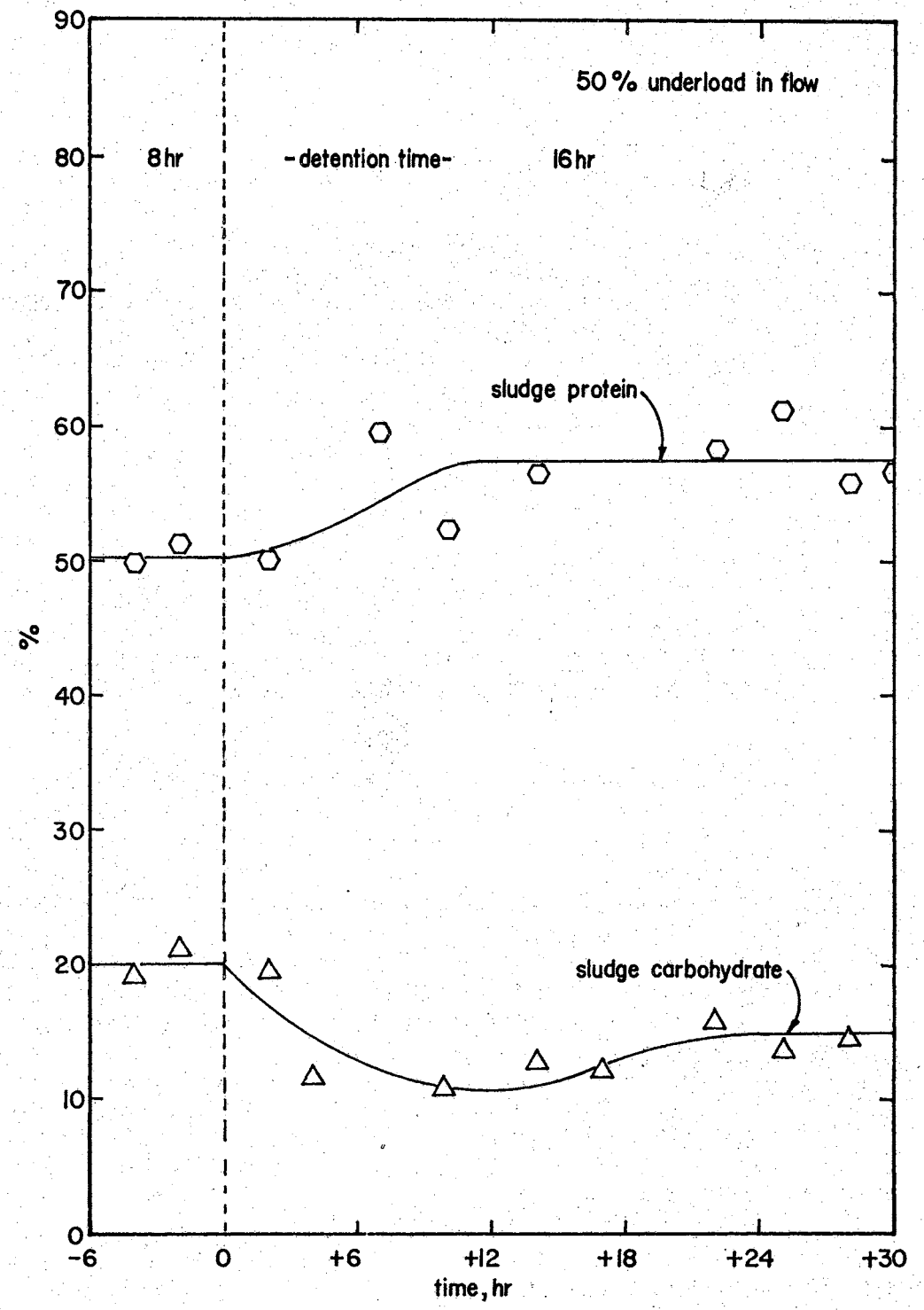

Figure 5 - Changes in sludge composition of a system shock loaded from a dilution rate of 0.125 hour^{-1} to 0.062 hour^{-1} (constant concentration).

evident increase in the protein and decrease in the carbohydrate content of the sludge.

Figure 6 shows the mildest shock (reduction in flow rate) that was applied, i.e., a change in dilution rate from 0.125 to 0.094 $hour^{-1}$ or detention time from eight to 10.67 hours. There was a slight increase in biological solids, a reversal of the trend established in response to the previous two shock loads (Figures 2 and 4). There was very little change in the filtrate COD and anthrone COD. There was some increase in the total cell protein which was parallel to the increase in biological solids concentration; very little change was noted with respect to the total cell carbohydrates. Figure 7 shows the percentage composition of sludge during this experiment. There was a small increase in the percentage protein, but no significant change in the percentage carbohydrate was noted.

b. Increase in Dilution Rate

In this series of experiments, the shock consisted of an increase in flow rate. Figure 8 shows the results observed when the flow rate was increased by 50 per cent causing a change in dilution rate from 0.125 to 0.188 $hour^{-1}$ or in detention time from eight hours to 5.33 hours. The biological solids fluctuated around an average value of 580 mg/l, and the changes in anthrone COD and COD of the filtrate were small. The decrease in total cell protein was seen to be at the expense of the cell carbohydrates. Figure 9 shows the percentages of protein and carbohydrates

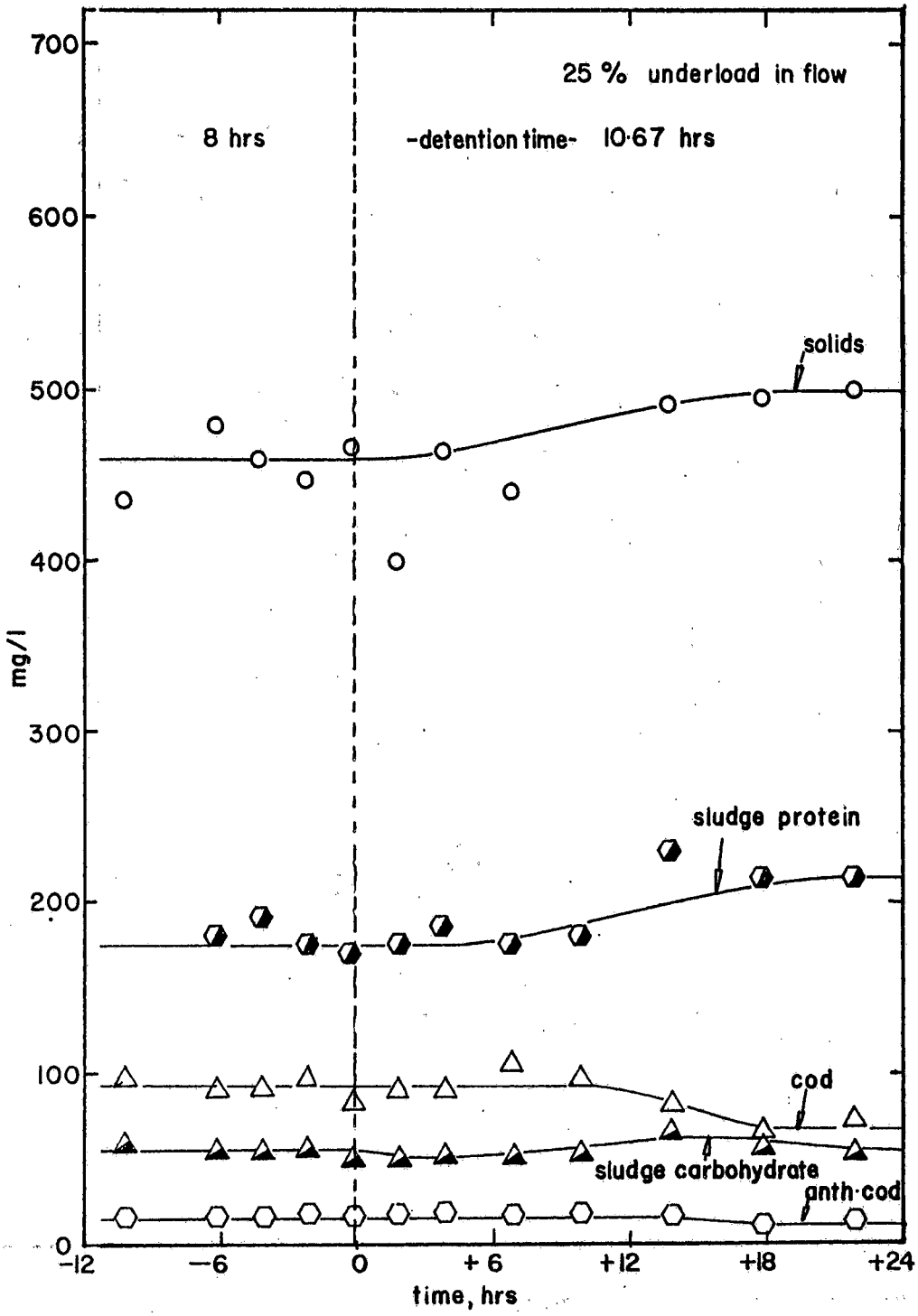

Figure 6 - Response of a steady state system shock loaded from a dilution rate of 0.125 hour^{-1} to 0.094 hour^{-1} (constant concentration).

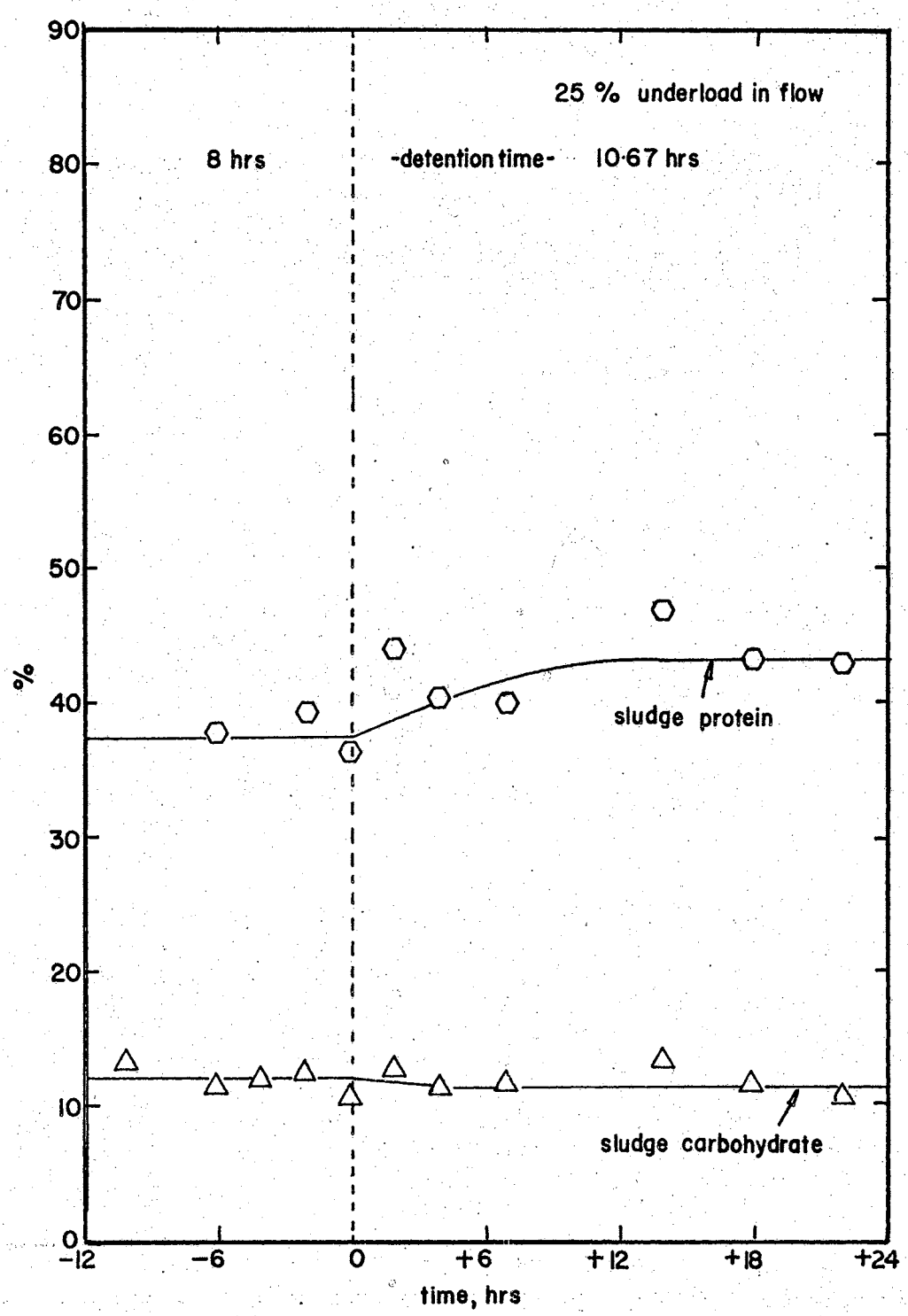

Figure 7 - Changes in sludge composition of a system shock loaded from a dilution rate of 0.125 hour^{-1} to 0.094 hour^{-1} (constant concentration).

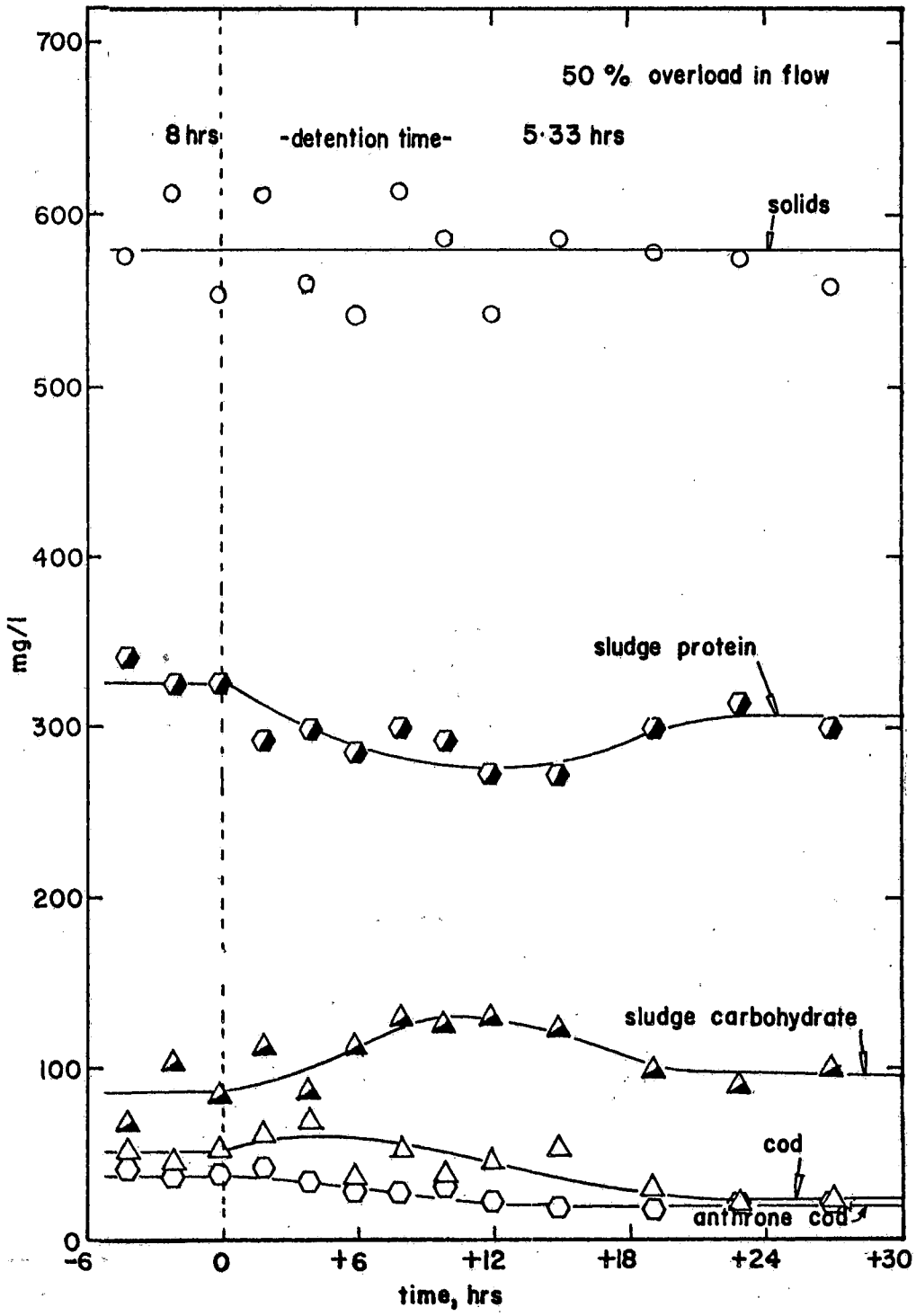

Figure 8 - Response of a steady state system shock loaded from a dilution rate of 0.125 hour^{-1} to 0.188 hour^{-1} (constant concentration).

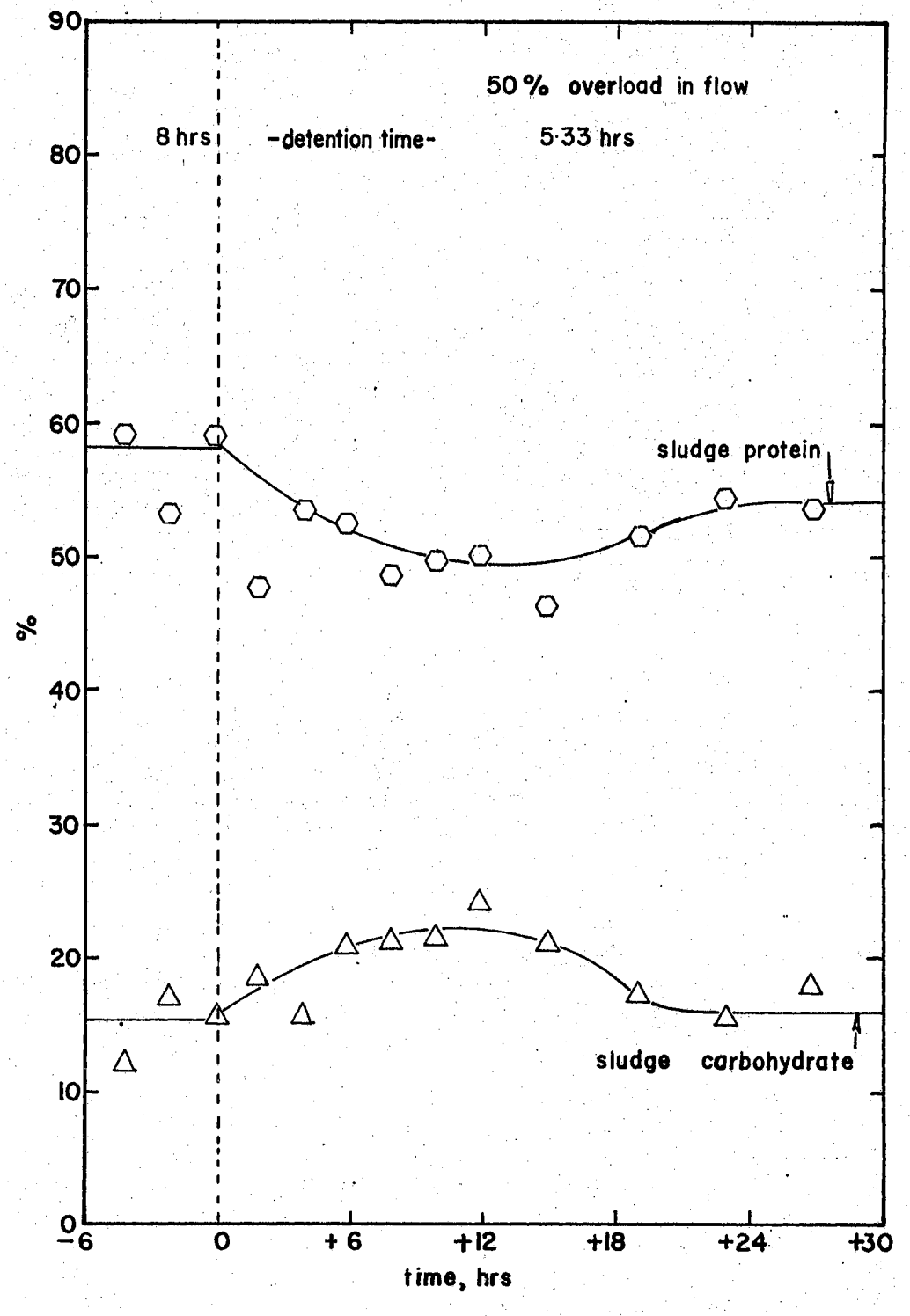

Figure 9 - Change in sludge composition of a system shock loaded from a dilution rate of 0.125 hour^{-1} to 0.188 hour^{-1} (constant concentration).

in the sludge. Since there was essentially no change in biological solids concentration, the percentage composition of protein and carbohydrate in the sludge followed the same trend as the absolute values in Figure 8.

Figure 10 shows the biochemical response of a system which was subjected to a hydraulic overload of 100 per cent, i.e., a change in dilution rate from 0.125 $hour^{-1}$ to 0.25 $hour^{-1}$ or in detention time from eight hours to four hours. There was a significant increase in biological solids concentration due to a change in either microbial predominance or yield coefficient. During the transient state there was some leakage of COD, but the effluent COD concentration returned to normal in approximately eight hours. However, the anthrone COD did not increase during the transient state; in fact, it decreased slightly, indicating that the COD leaving the reactor consisted of metabolic intermediates which were noncarbohydrate in nature. The cell protein decreased initially, but later returned to a level slightly higher than the previous steady state concentration. There was some small increase in the cellular carbohydrate. Figure 11 shows the sludge composition for this experiment. In general, there was some decrease in the percent protein, but the change in carbohydrate level was very small.

Figure 12 shows the response when the system was overloaded by 150 per cent, by a change in dilution rate from 0.125 $hour^{-1}$ to 0.313 $hour^{-1}$ or in detention time from eight hours to 3.2 hours. It is seen that the efficiency of

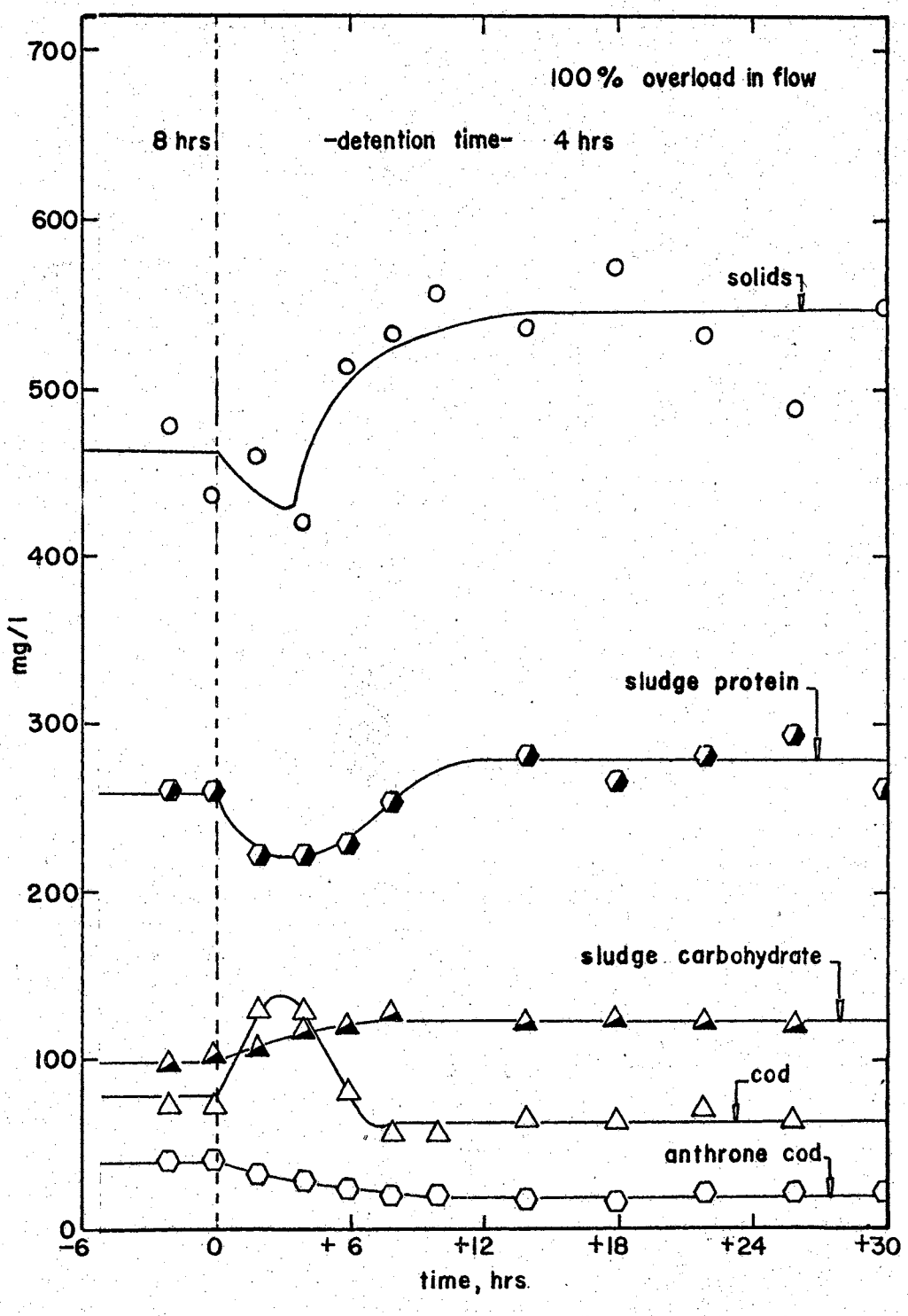

Figure 10 - Response of a system shock loaded from a dilution rate of 0.125 hour^{-1} to 0.25 hour^{-1} (constant concentration).

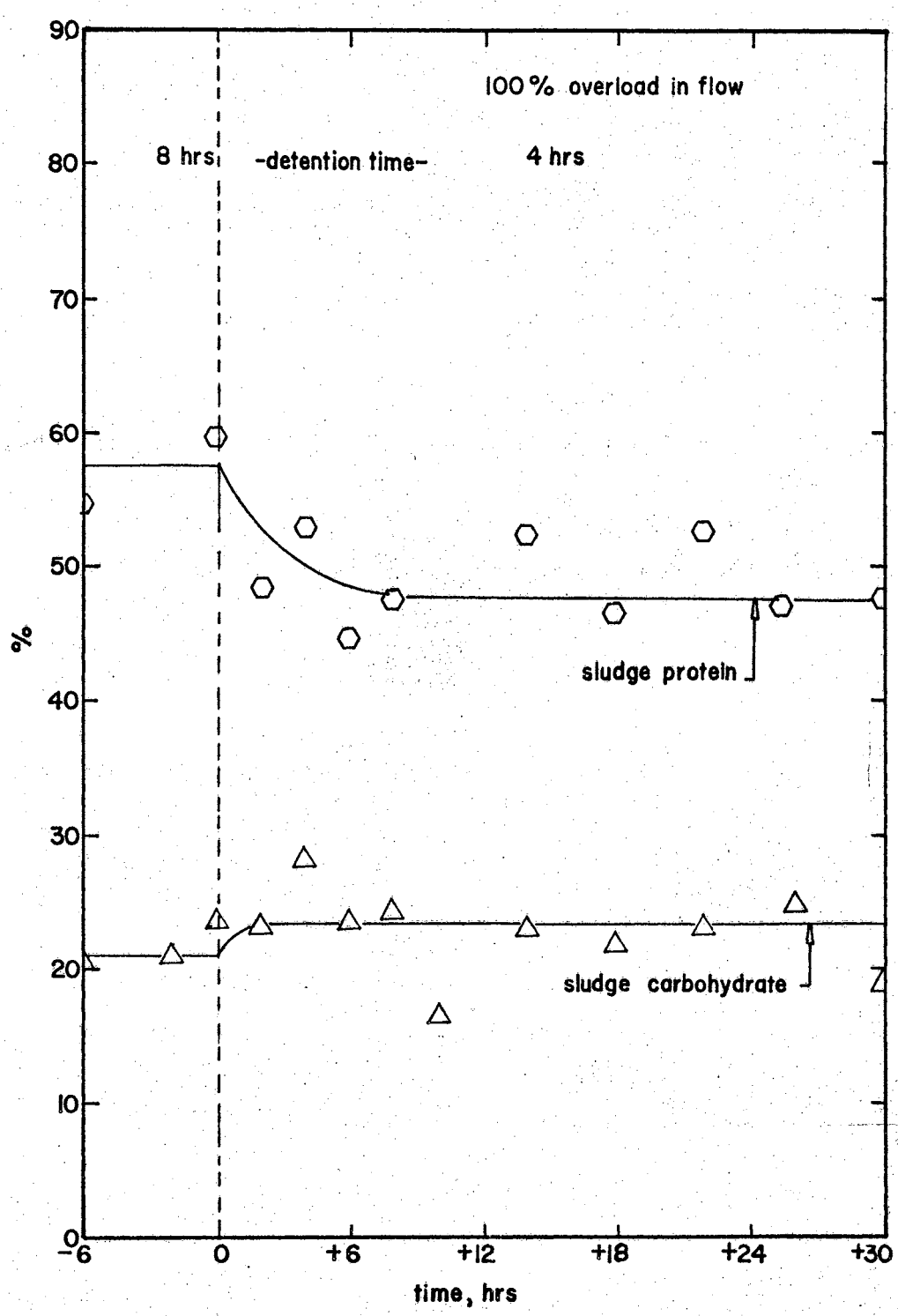

Figure 11 - Changes in sludge composition of a system shock loaded from a dilution rate of 0.125 hour^{-1} to 0.25 hour^{-1} (constant concentration).

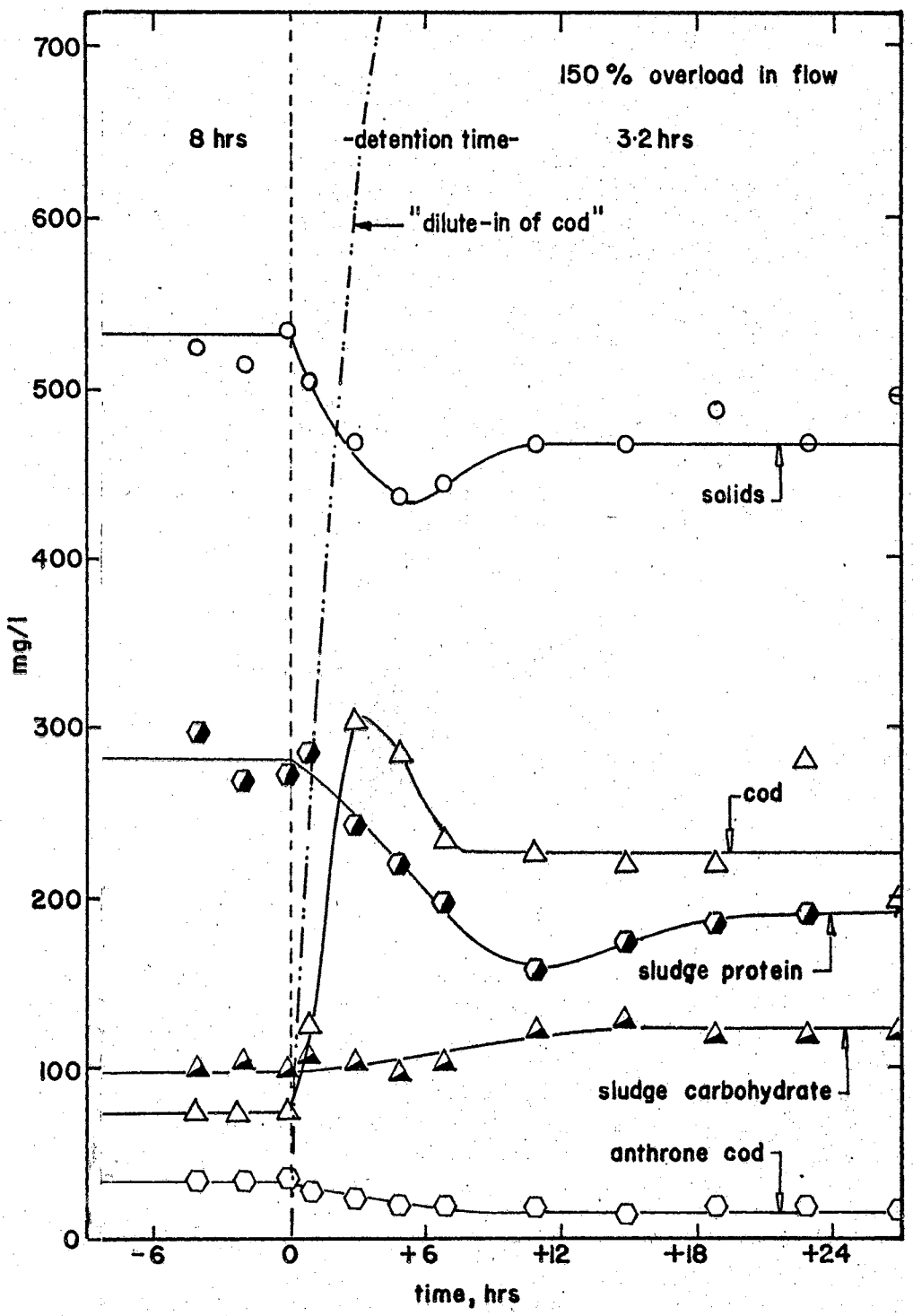

Figure 12 - Response of a system shock loaded from a dilution rate of 0.125 hour^{-1} to 0.313 hour^{-1} (constant concentration).

the system was disrupted severely. The solids level dropped from approximately 530 mg/l prior to the shock to 430 mg/l during the transient, and then recovered to 470 mg/l after the shock. There was a corresponding change in the effluent COD which resulted in an increase from 80 mg/l to approximately 230 mg/l. It is to be noted that during the transient conditions immediately after the shock, the minimum biological solids concentration and the maximum effluent COD were greater than their corresponding values at the final steady state levels. There was considerable decrease in the total cell protein and some increase in cellular carbohydrate. The "dilute-in" curve indicates the COD that would have been present if there were no biochemical reactions occurring in the reactor. However, the actual effluent COD does not coincide with this dilute-in curve, indicating the occurrence of biochemical changes resulting from the metabolism of the influent organic matter inside the aerator. It can also be seen that the anthrone COD of the effluent did not increase with the total COD, indicating conversion of glucose to metabolic intermediates and/or end products of a noncarbohydrate nature. Figure 13 shows the changes in sludge composition during this experiment. There was an appreciable reduction in percent protein and a moderate increase in percent carbohydrate of the sludge.

Figure 14 shows the results for an even more severe shock loading condition, i.e., a 200 per cent overload in flow rate, causing a change in dilution rate from 0.125

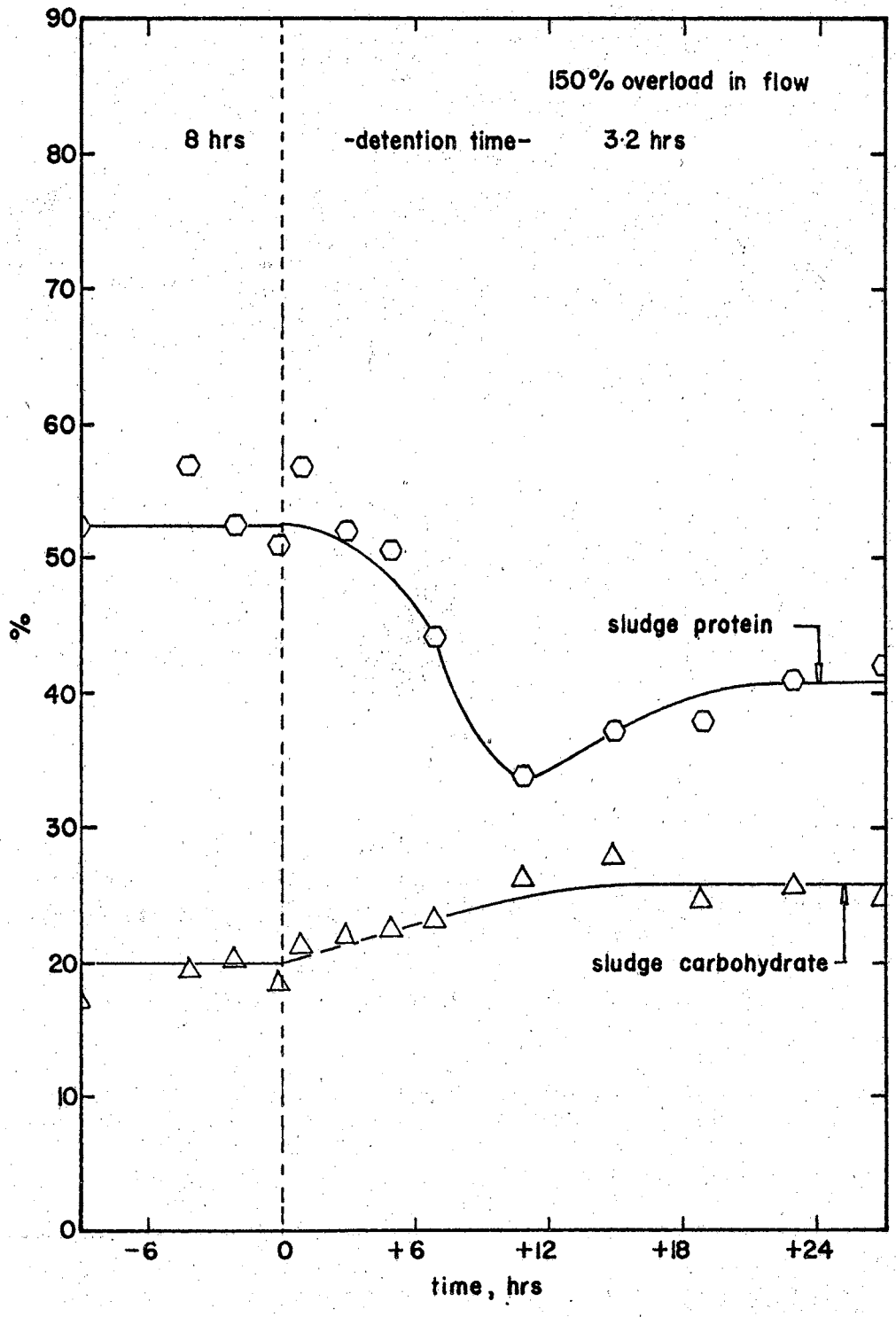

Figure 13 - Change in sludge composition of a system shock loaded from a dilution rate of 0.125 hour^{-1} to 0.313 hour^{-1} (constant concentration).

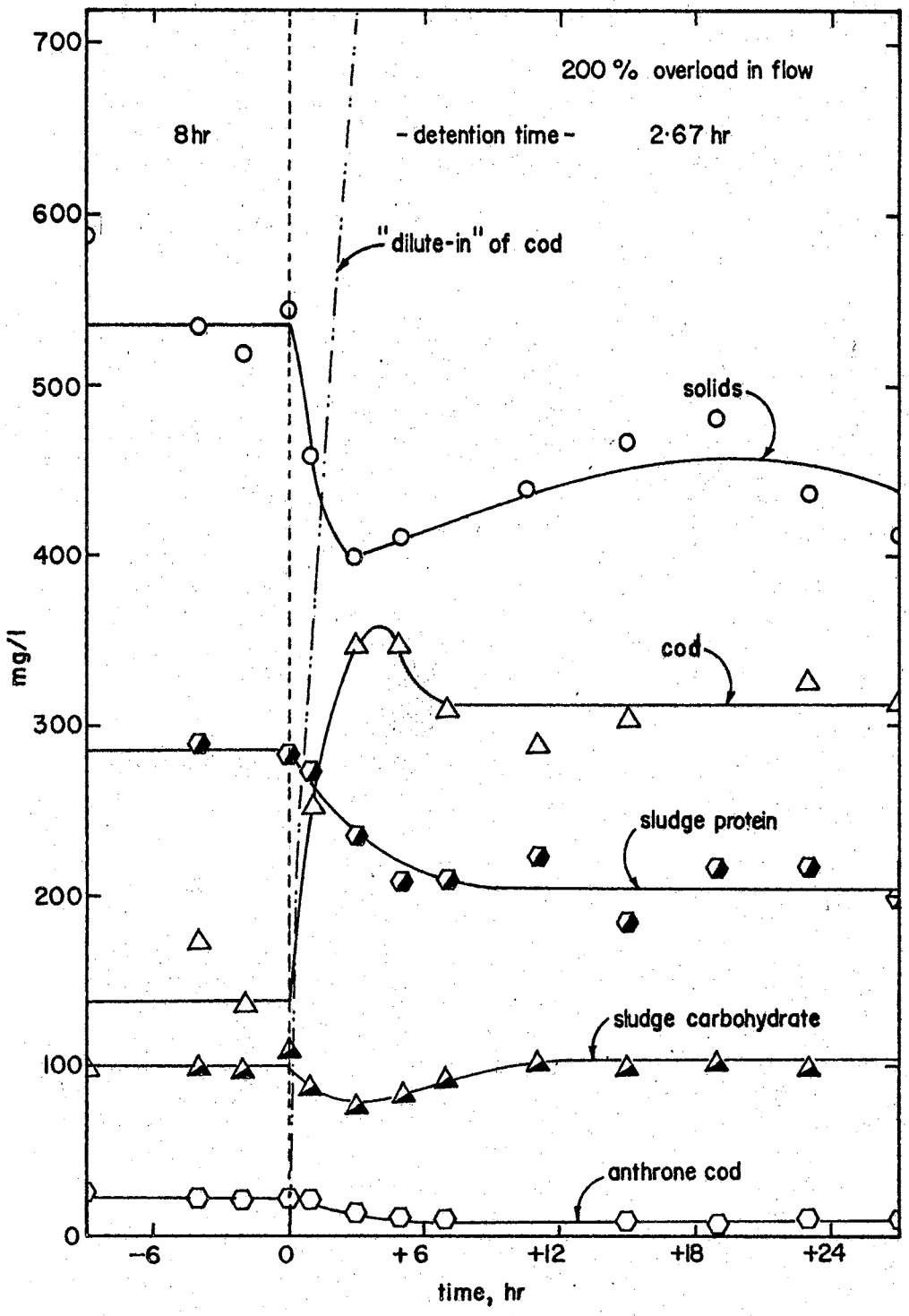

Figure 14 - Response of a steady state system shock loaded from a dilution rate of 0.125 hour^{-1} to 0.375 hour^{-1} (constant concentration).

hour^{-1} to 0.375 hour^{-1} or in detention time from eight hours to 2.67 hours. The biological solids level dropped more severely in this experiment than it did in the experiment shown in Figure 12. There was a slow recovery in the solids level, followed by another decline. The results indicate that the biological solids level was oscillating and had not reached a steady state during the experimental period. The effluent COD increased from approximately 140 mg/l to a transient peak value of 360 mg/l, and then approached a new steady state level of approximately 310 mg/l. As in the foregoing experiment, the bulk of the COD in the effluent consisted of metabolic products other than carbohydrates. Comparing the effluent COD curve and the COD dilute-in curve during the transient state, it is evident that more COD was being metabolized than was leaking from the reactor. There was considerable reduction in total cell protein; however, the cellular carbohydrate showed less change. Figure 15 shows that during this experiment the percentage protein of the cells dropped considerably, but change in percent carbohydrate was small.

Figure 16 shows the response to the most severe hydraulic shock load applied. The change in flow rate represented a 250 per cent overload, resulting in a change in dilution rate from 0.125 hour^{-1} to 0.437 hour^{-1}, or in detention time from eight hours to 2.29 hours. In this case, the biological solids were "diluted out" severely from approximately 540 mg/l to a new steady state value of

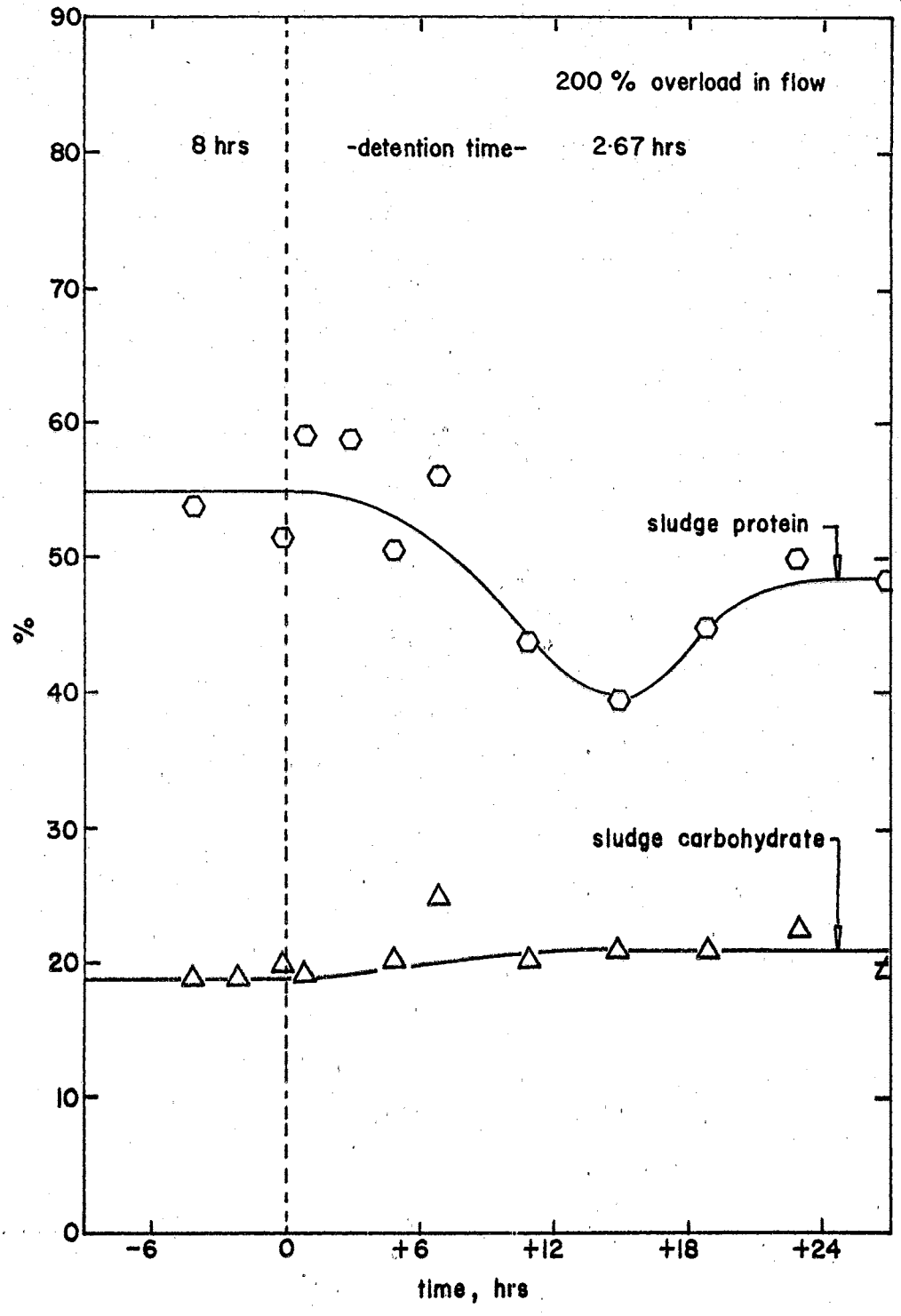

Figure 15 - Change in sludge composition of a system shock loaded from a dilution rate of 0.125 hour^{-1} to 0.375 hour^{-1} (constant concentration).

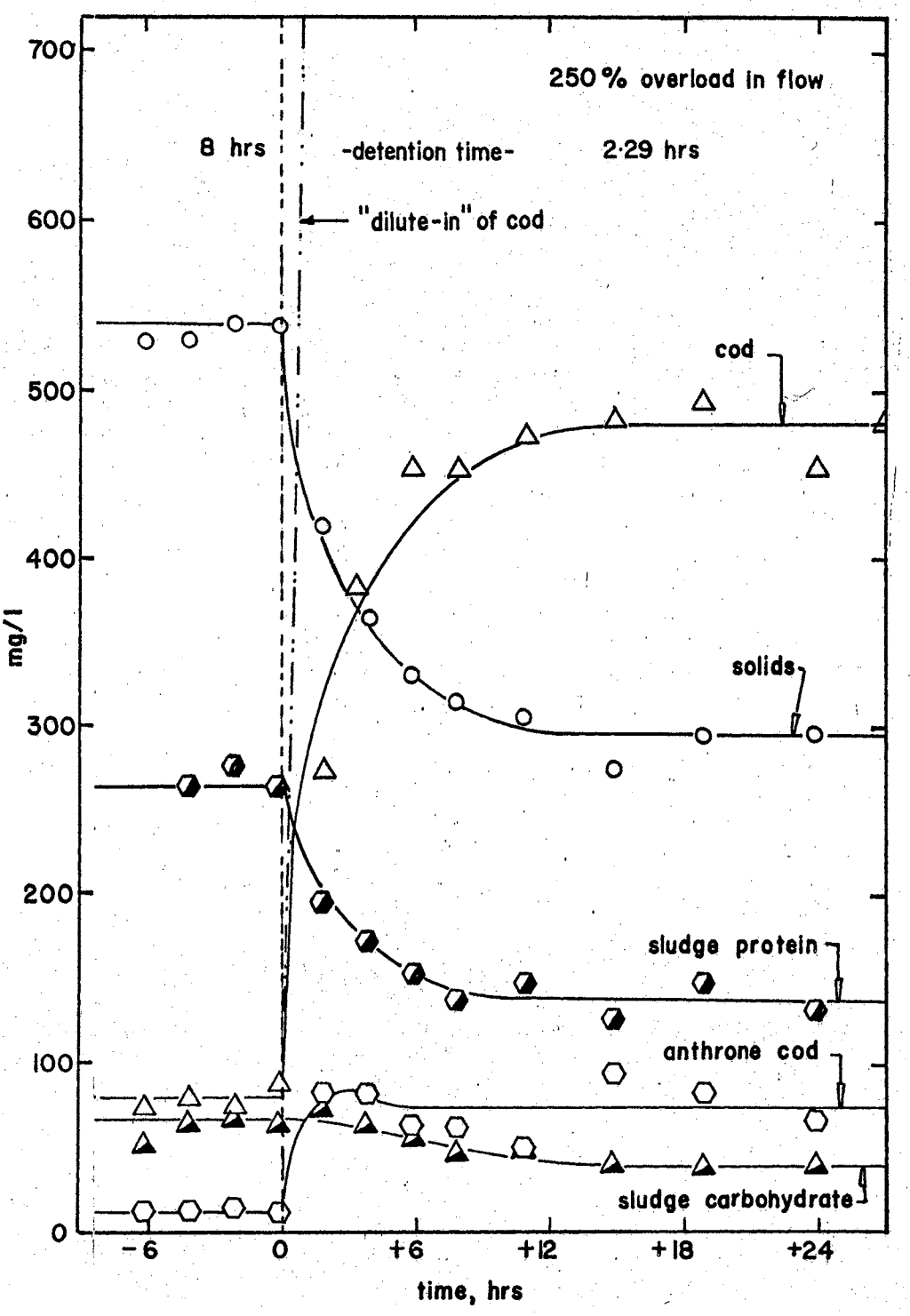

Figure 16 - Response of a system shock loaded from a dilution rate of 0.125 hour^{-1} to 0.437 hour^{-1} (constant concentration).

300 mg/l. The effluent COD increased from approximately 80 mg/l to 480 mg/l at the new steady state. Even though the influent COD dilute-in curve was close to the effluent COD curve immediately following the shock, the amount of COD metabolized increased with time after the shock. The total cell protein decreased almost in parallel to the decrease in the biological solids concentration. The total carbohydrate concentration of the sludge also decreased. There would appear to be little doubt that the system was operating close to the maximum growth rate and permanent cell dilute-out was occurring, i.e., the system was operating on the sloping portion of the "dilute-out" curve. Even though the anthrone COD showed some increase, the release of metabolic intermediates and/or end products was extremely high. Figure 17 shows the changes in sludge parameters during the experiment. The percentage content of protein in the sludge decreased; however, the decrease was not comparable with those of the previous two shock loads. There was some increase in the percent cellular carbohydrate. The percent RNA content was also seen to increase as a result of the more rapid growth rate imposed upon the cells.

On the whole, an increase in flow rate caused more severe disturbance to the system than did a reduction in flow rate. It is to be emphasized that in all of these experiments the glucose concentration in the feed was maintained at 1000 mg/l. Therefore it can be seen that an increase in flow rate caused an automatic increase in

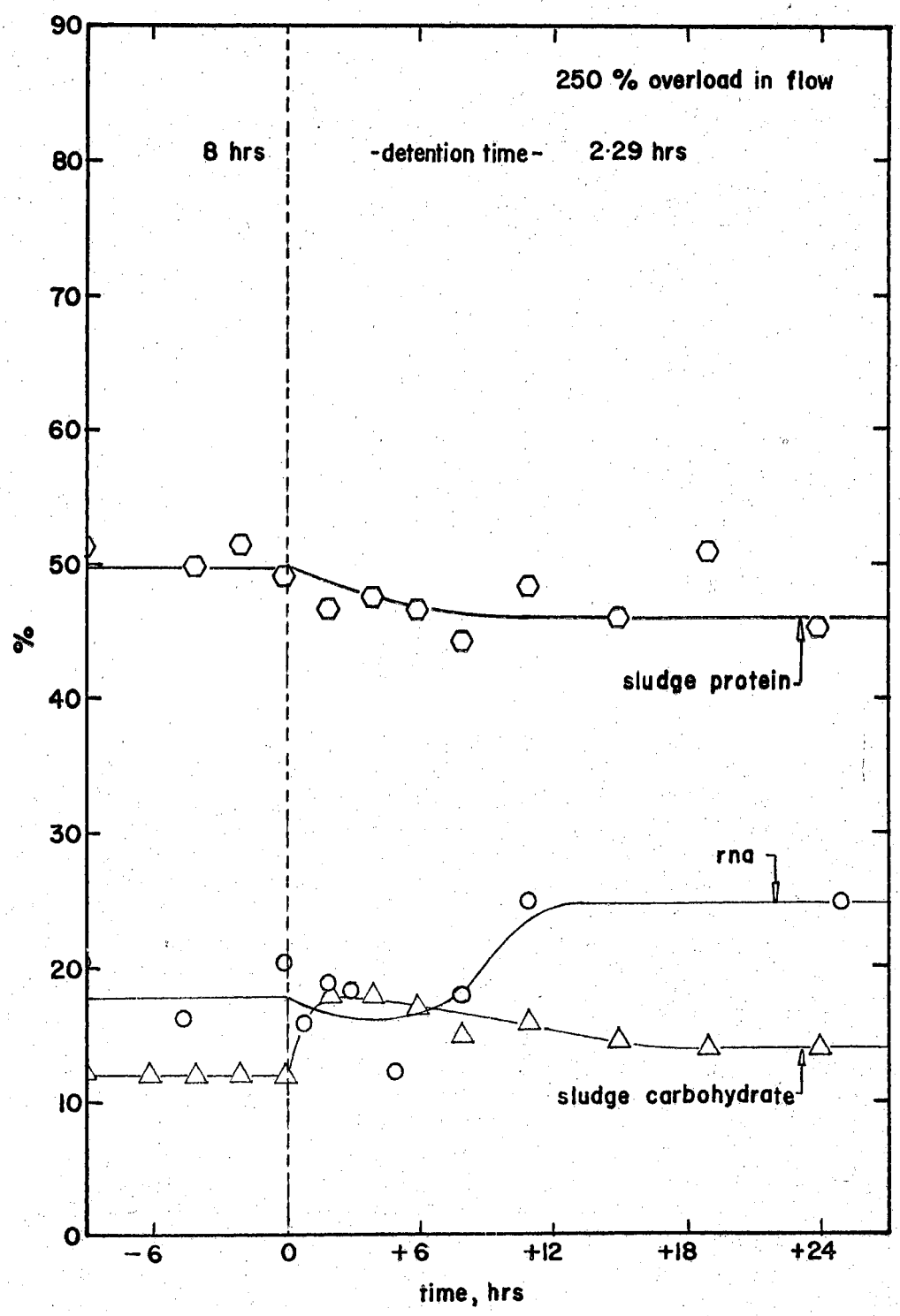

Figure 17 - Changes in sludge composition of a system shock loaded from a dilution rate of 0.125 hour^{-1} to 0.437 hour^{-1} (constant concentration).

organic loading, and a decrease in flow rate resulted in a parallel decrease in organic loading, (i.e., lbs BOD or COD/unit volume/day).

2. Constant Daily Organic Loading Conditions

The second series of experiments on hydraulic shock loads was conducted at "constant daily organic loading conditions" in contrast to the previous series in which the feed concentration, rather than the loading, was constant. The constant organic loading was 198 lbs COD/1000 cu ft of aerator volume/day both before and after the shock. The steady state conditions for all experiments were based on 1000 mg/l glucose in the influent, and a detention time of eight hours in the aerator before the shock loads were applied.

a. Decrease in Dilution Rate

Figure 18 shows the response when the system was shock loaded from a dilution rate of 0.125 hour^{-1} (eight-hour detention time) and 1000 mg/l feed glucose concentration, to a dilution rate of 0.031 hour^{-1} (thirty-two hour detention time) and 4000 mg/l glucose in the feed. It is seen that the biological solids level increased from 400 mg/l to approximately 1150 mg/l. The transient state response of biological solids to the increase in substrate concentration was very rapid. There did not appear to be a lag or adjustment period before the solids concentration in the aerator began to rise in response to the increase in glucose concentration. The filtrate COD rose from a steady state

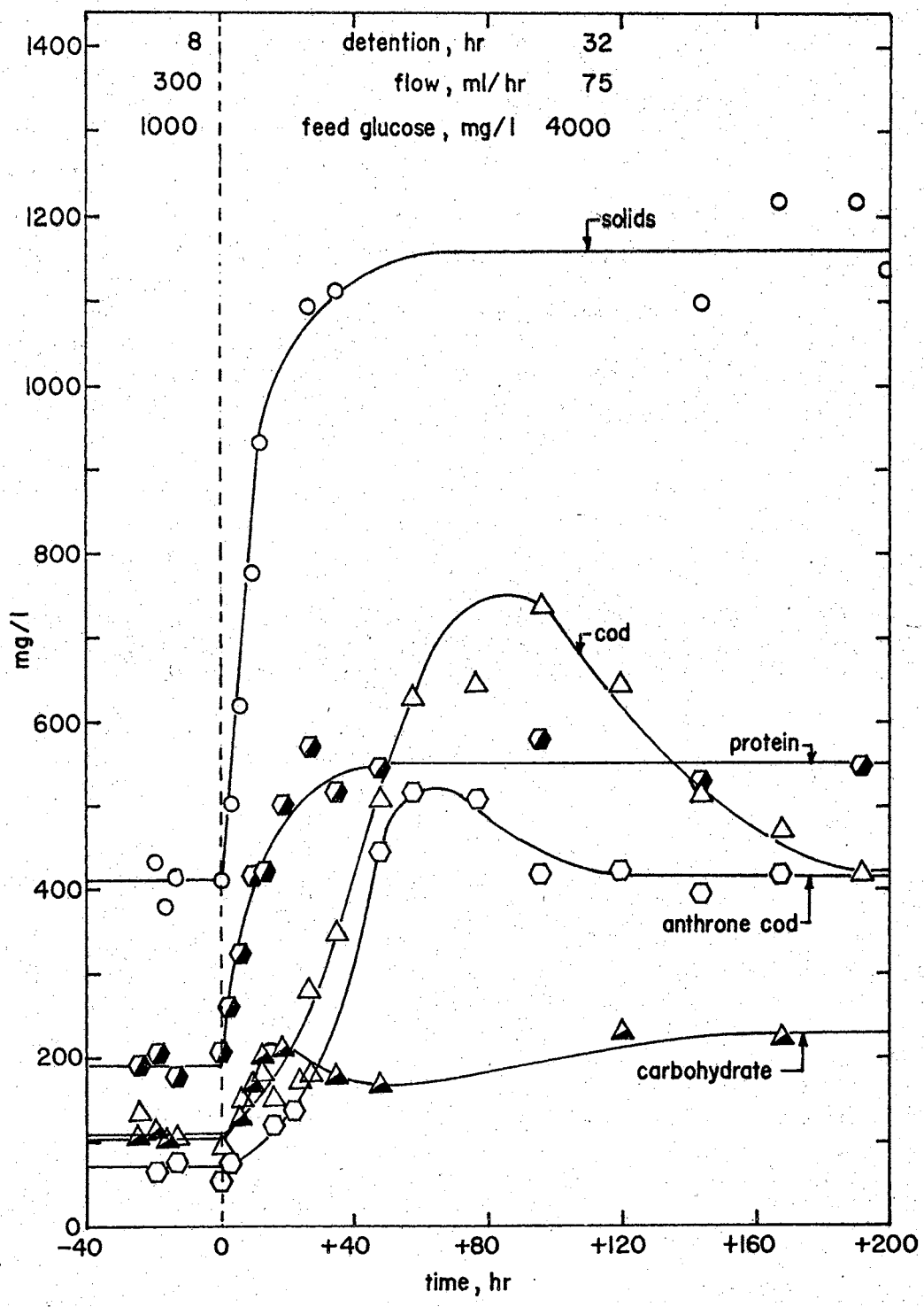

Figure 18 - Response of a system shock loaded from a dilution rate of 0.125 hour^{-1} to 0.031 hour^{-1} (constant loading).

value of nearly 110 mg/l to a transient peak value of approximately 760 mg/l at 85 hours (2.7 detention times) after the shock, and thereafter decreased. Initially, the anthrone COD curve parallelled the COD curve, but after sixty hours (1.9 detention periods) it began to decrease. It is interesting to note that the metabolic intermediates which were produced were apparently metabolized after the system became acclimated. The high residual COD due to carbohydrates (anthrone test) at the end of the experiment is noteworthy, and it shows that the system could not totally recover to its former low level of effluent carbohydrates even after 200 hours. There was a substantial buildup of cellular protein, and some increase in the cellular carbohydrate concentration. Figure 19 shows the changes in various sludge components during this experiment. The percent protein decreased slightly; however, the percentage of cellular carbohydrates increased appreciably. There was a decrease in the RNA content of the sludge, but some increase in the DNA content. Figure 20 shows the elemental analysis of sludge (carbon, hydrogen, and nitrogen). It is seen that there was no significant change with respect to the percent carbon, nitrogen, and hydrogen content of the sludge.

Figure 21 shows the biochemical response of a system shock loaded from a dilution rate of 0.125 $hour^{-1}$ (detention time eight hours) and 1000 mg/l feed glucose to a dilution rate of 0.062 $hour^{-1}$ (detention time sixteen hours) and 2000 mg/l feed glucose. This caused an increase in

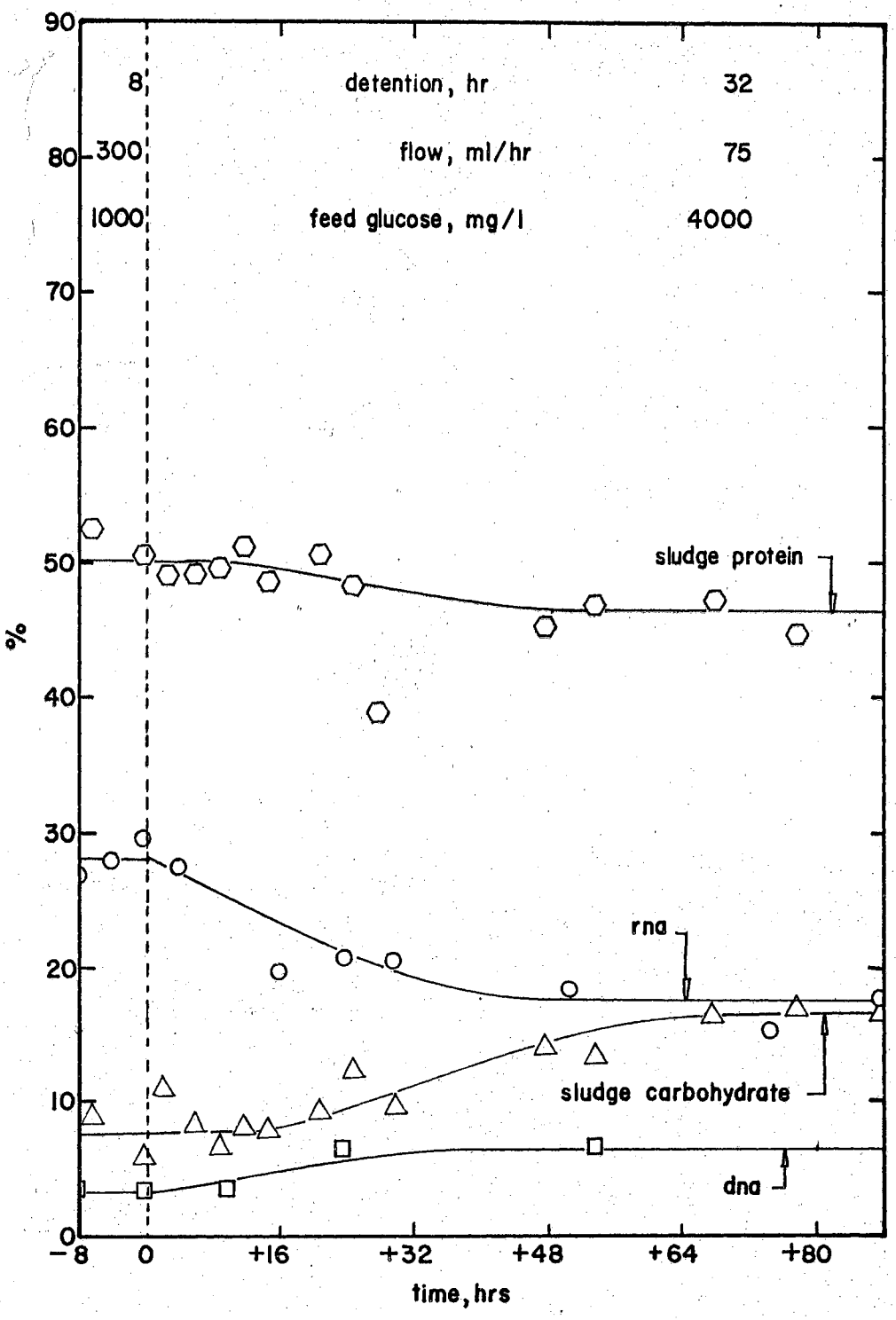

Figure 19.- Change in sludge composition of a system shock loaded from a dilution rate of 0.125 hour^{-1} to 0.031 hour^{-1} (constant loading).

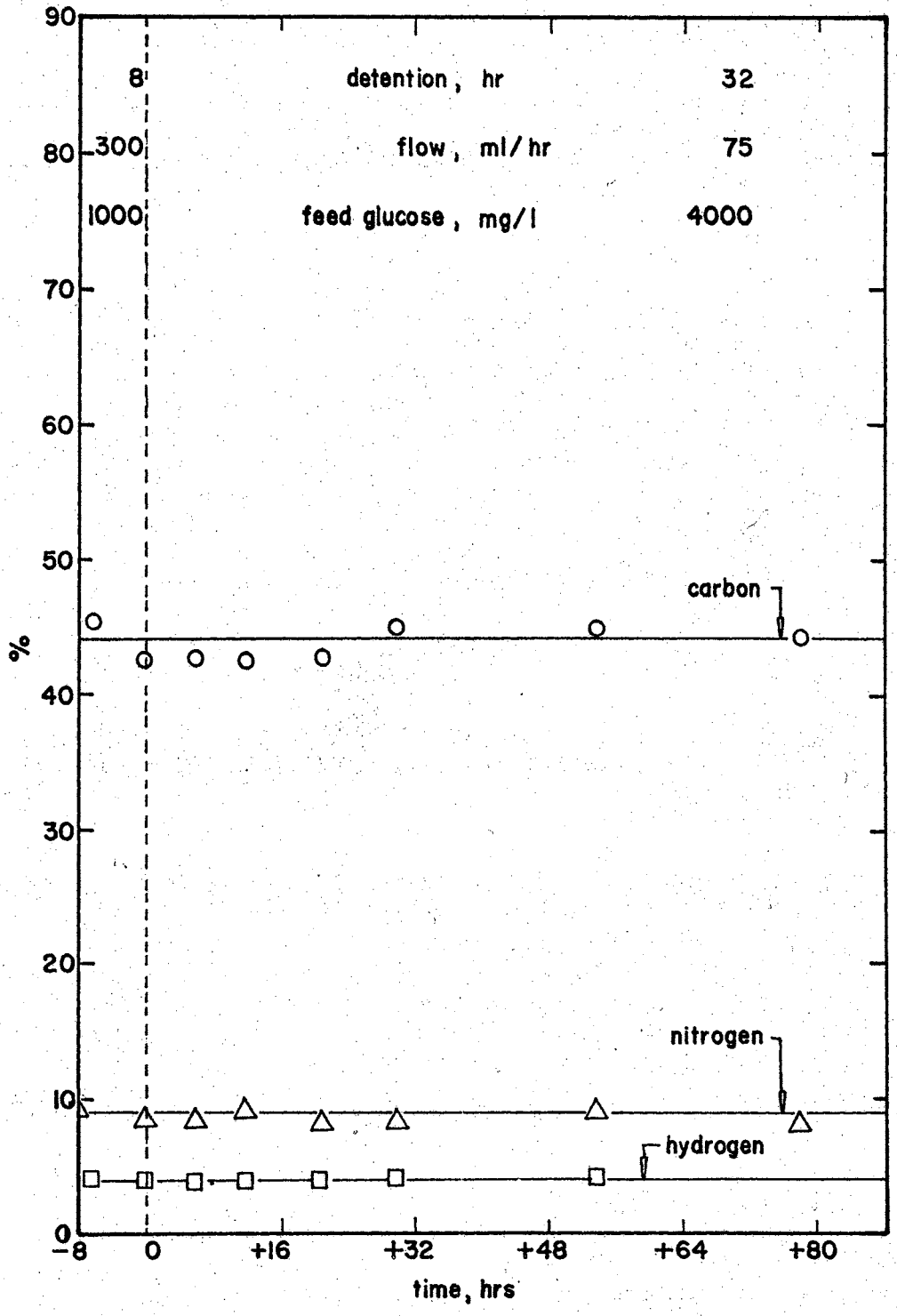

Figure 20 - Changes in elemental composition of sludge from a system shock loaded from a dilution rate of 0.125 hour^{-1} to 0.031 hour^{-1} (constant loading).

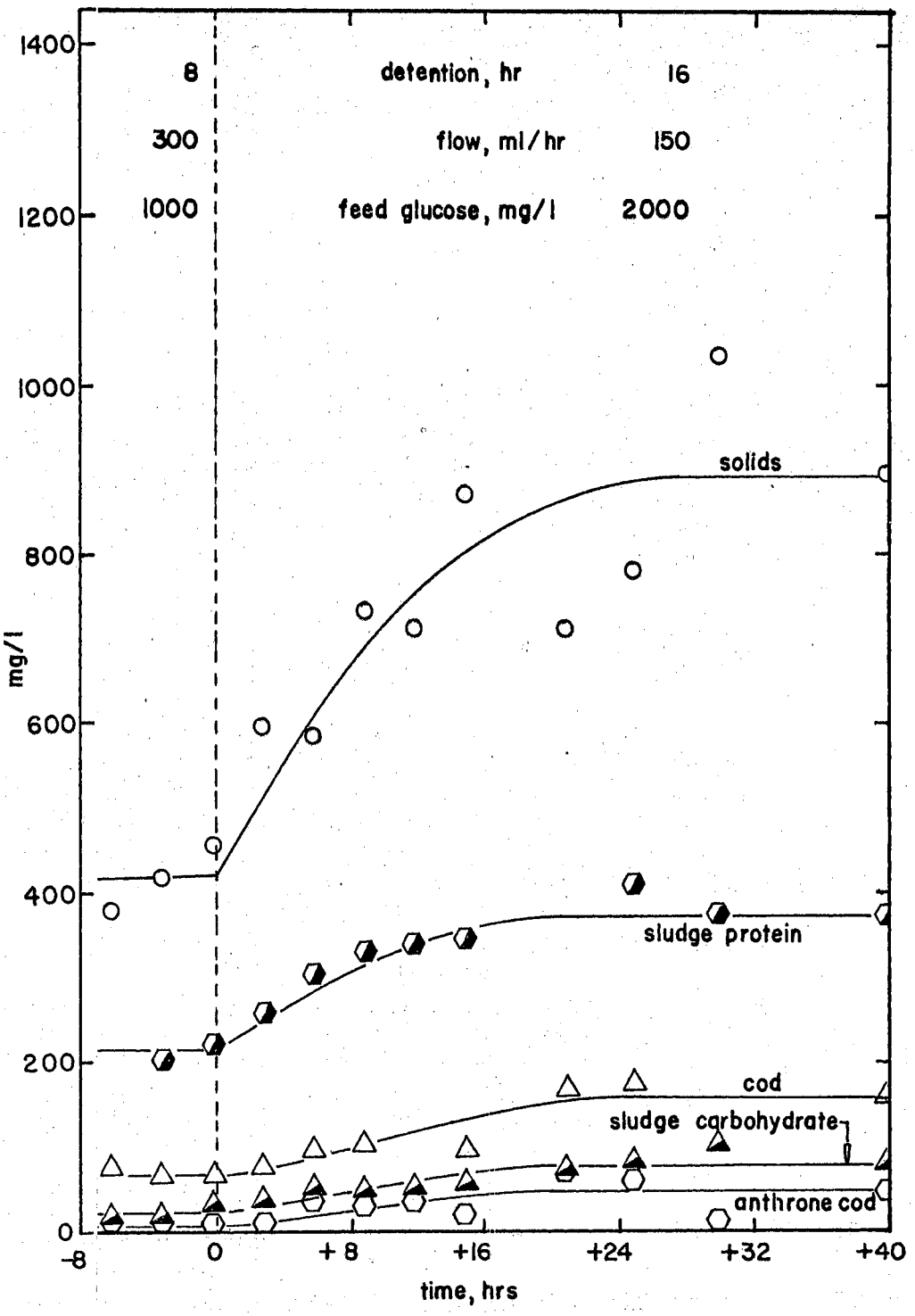

Figure 21 - Response of a steady state system shock loaded from a dilution rate of 0.125 hour^{-1} to 0.062 hour^{-1} (constant loading).

biological solids from approximately 420 mg/l to nearly 900 mg/l at the new steady state. The response of the filtrate COD was considerably better than in the previous case (Figure 18). There was an increase from 70 mg/l to approximately 160 mg/l, indicating only very slight decrease in COD removal efficiency. The anthrone COD increased from a low value of 8 mg/l to approximately 50 mg/l at the new steady state. The total cellular protein increased steadily from nearly 210 mg/l to approximately 380 mg/l, while the cellular carbohydrate concentration increased from 25 mg/l to nearly 80 mg/l. Figure 22 shows the protein and carbohydrate during the experiment. There was considerable reduction in protein content. The carbohydrate content increased as a result of the shock load. Comparing this figure with Figure 5, which shows the parallel case under constant concentration conditions, it can be seen that the two types of hydraulic shocks produced opposite effects on cell composition.

Figure 23 shows the mildest shock load applied in this series. The dilution rate was changed from 0.125 $hour^{-1}$ to 0.094 $hour^{-1}$. The corresponding change in detention time was from eight hours to 10.67 hours. To maintain constant loading conditions, the feed glucose concentration was changed from 1000 mg/l to 1333 mg/l. It is seen that while there was no great amount of COD leakage during the transient, the final steady state value after the shock was significantly higher. The solids and anthrone COD were

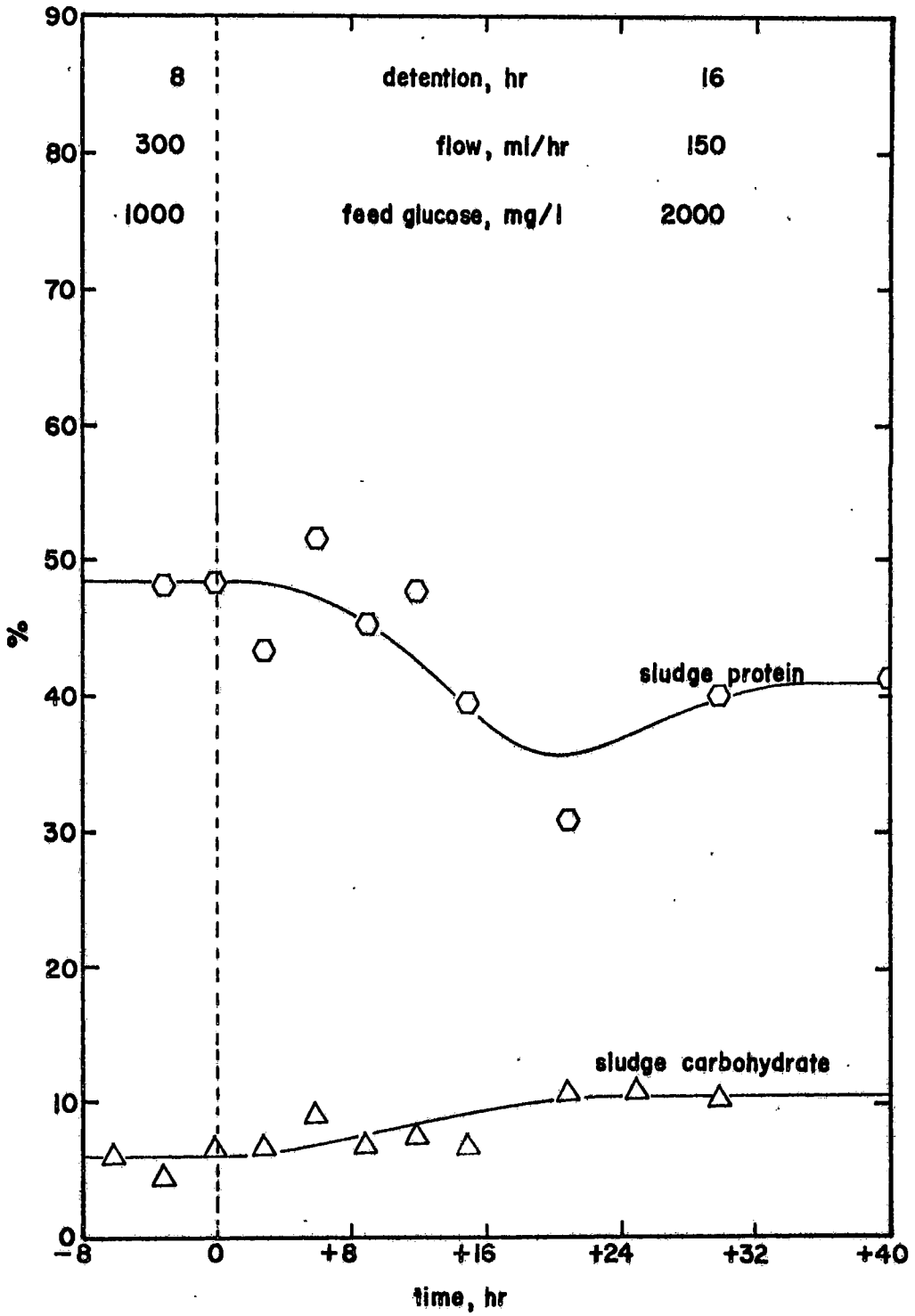

Figure 22 - Change in sludge composition of a system shock loaded from a dilution rate of 0.125 hour^{-1} to 0.062 hour^{-1} (constant loading).

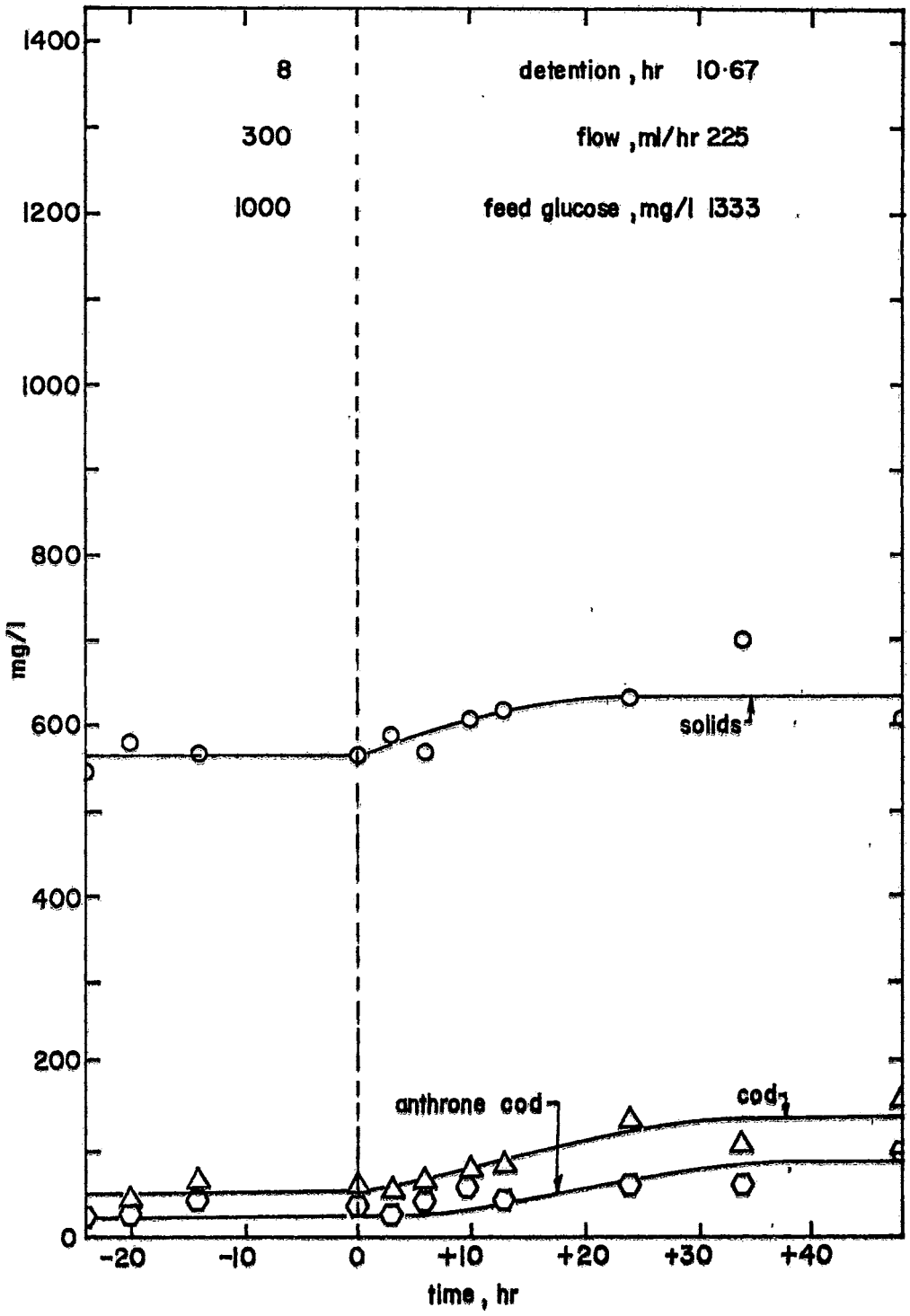

Figure 23 - Response of a system shock loaded from a dilution rate of 0.125 hour^{-1} to 0.094 hour^{-1} (constant loading).

also slightly higher at the new steady state, compared to the old steady state values. Figure 24 shows that there was very little variation in the percent protein and carbohydrate of the sludge.

b. Increase in Dilution Rate

This series of experiments includes those in which the flow rates were increased and the glucose concentrations in the feed reduced to maintain constant daily organic loading conditions. Figure 25 shows the response of a system subjected to a change in dilution rate from 0.125 $hour^{-1}$ (detention time eight hours), and 1000 mg/l feed glucose to a dilution rate of 0.188 $hour^{-1}$ (detention time 5.33 hours) and 667 mg/l feed glucose. There was no significant change in filtrate COD or anthrone COD. The only significant change was the drop in biological solids concentration which occurred in response to the decrease in feed concentration.

Figure 26 shows the response of a system which was shock loaded from a dilution rate of 0.125 $hour^{-1}$ (detention time eight hours) and feed glucose concentration 1000 mg/l to a dilution rate of 0.25 $hour^{-1}$ (detention time four hours) and feed glucose concentration of 500 mg/l. There was considerable fluctuation in biological solids concentration during the transient and the steady state value after the shock was nearly 220 mg/l compared to the value of 380 mg/l before the shock. There was a slight reduction in filtrate COD from 125 mg/l to nearly 80 mg/l, and there was a corresponding reduction in the anthrone COD concentration. It

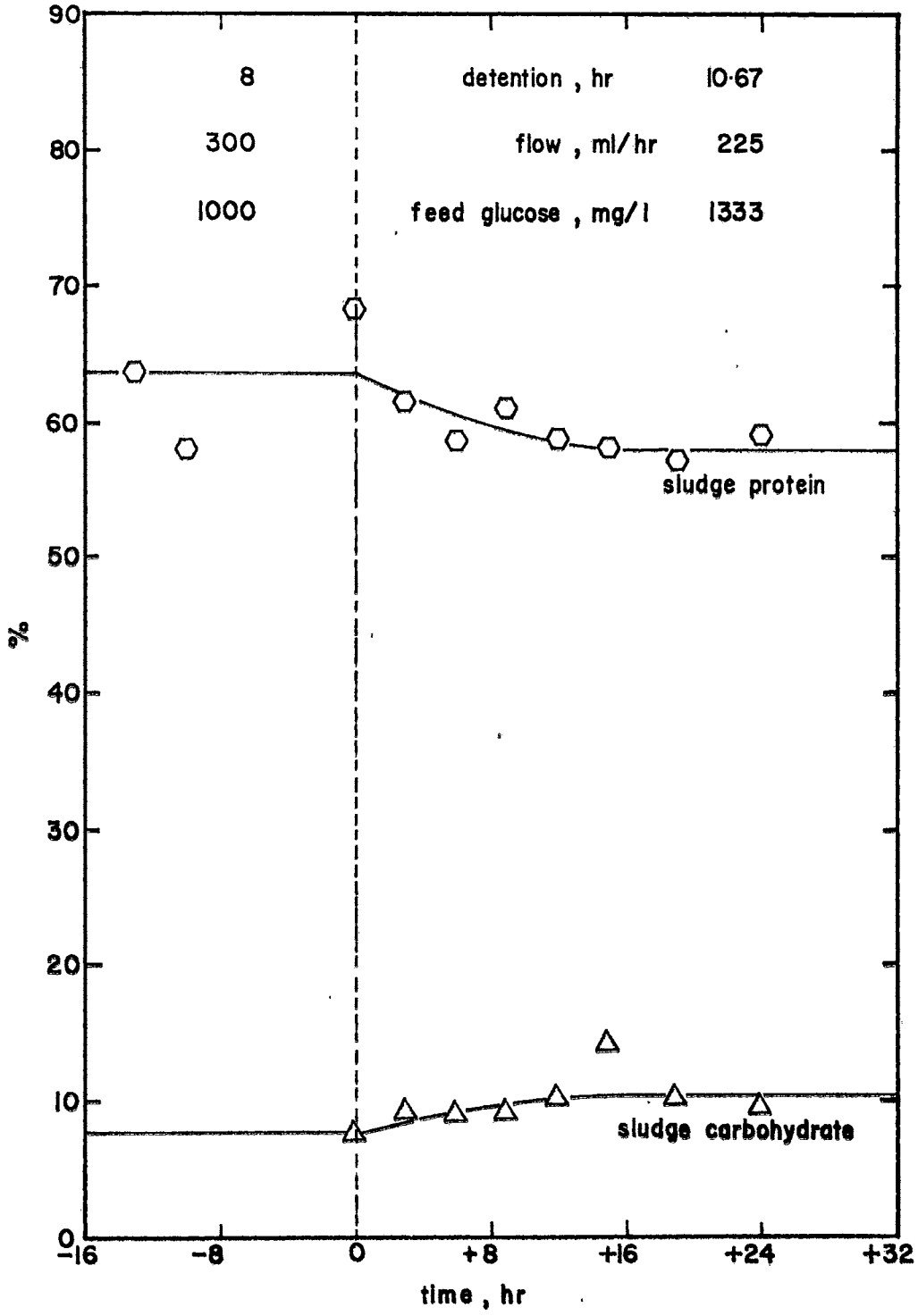

Figure 24 - Change in sludge composition of a system shock loaded from a dilution rate of 0.125 hour^{-1} to 0.094 hour^{-1} (constant loading).

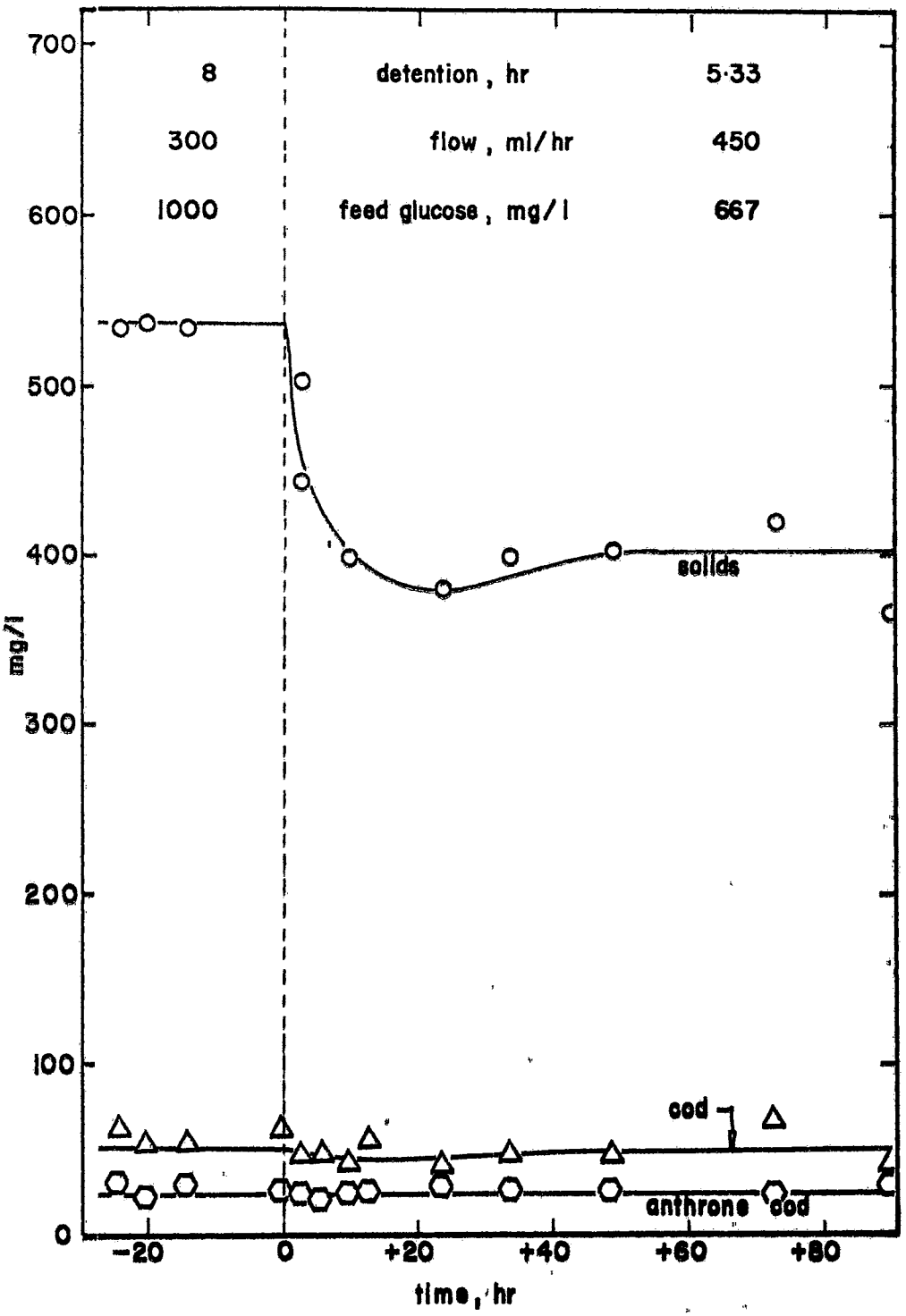

Figure 25 - Response of a system shock loaded from a dilution rate of 0.125 hour^{-1} to 0.188 hour^{-1} (constant loading).

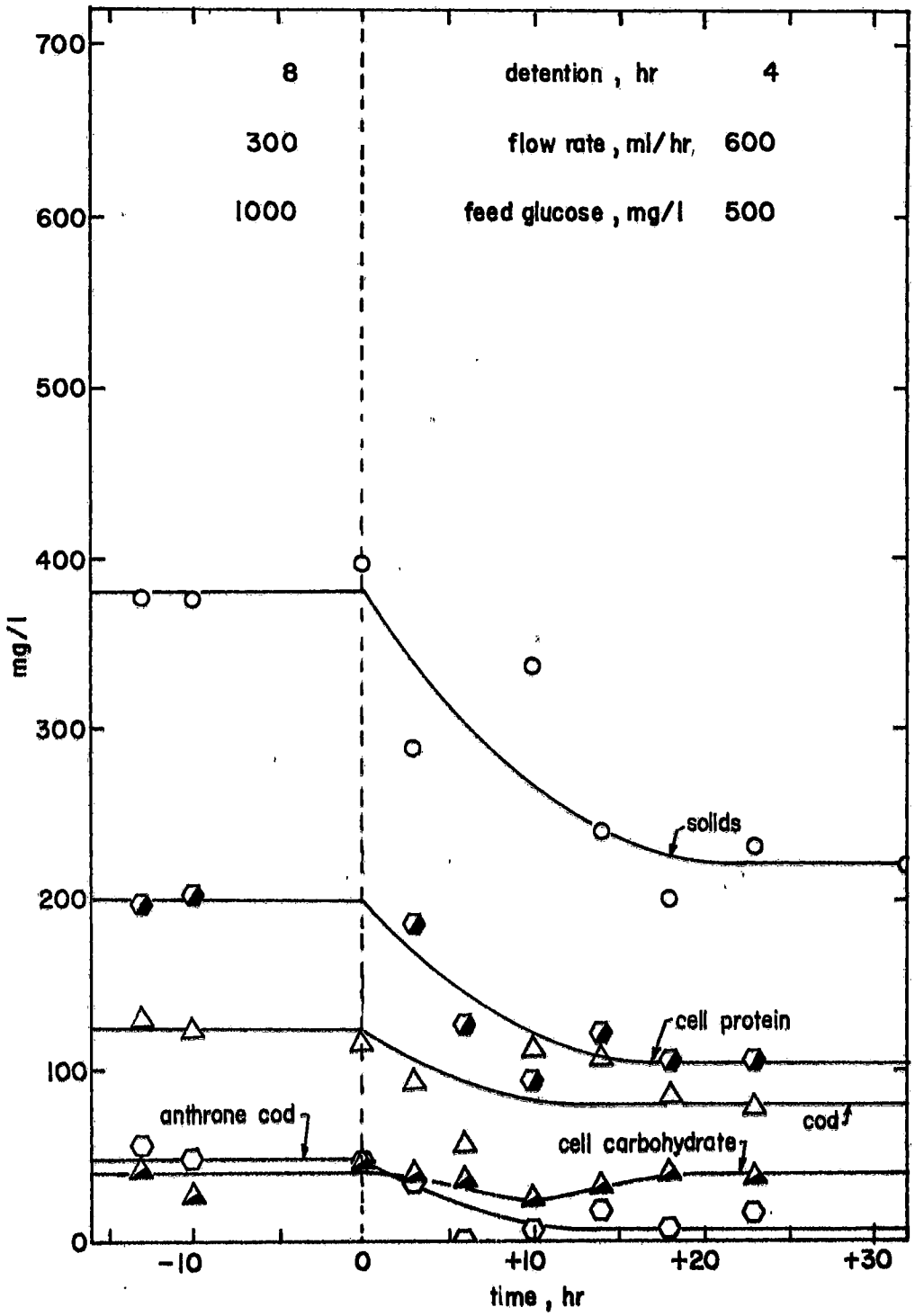

Figure 26 - Response of a system shock loaded from a dilution rate of 0.125 hour^{-1} to 0.25 hour^{-1} (constant loading).

is observed that the total protein was reduced in accordance with the solids concentration. However, the change in total cellular carbohydrate concentration was less significant. These sludge parameters expressed as percentages are shown in Figure 27. There was a significant reduction in percent protein, and there was an appreciable increase in the percent carbohydrate content of the cells. These results agree with those of Figure 11, where data for a similar hydraulic shock, but under constant concentration conditions, are given. It should be recalled that the data for these two different types of shock loads did not agree when the common factor was a reduction in flow rate.

Figure 28 shows a rather severe shock load; the dilution rate was changed from 0.125 hour^{-1} to 0.313 hour^{-1}. The parallel change in detention time was from eight hours to 3.2 hours, and in feed glucose concentration from 1000 mg/l to 400 mg/l (to maintain constant loading conditions). The biological solids concentration decreased from 300 mg/l to approximately 180 mg/l. The filtrate COD is also seen to have decreased from 150 mg/l to nearly 90 mg/l. There was a large reduction in anthrone COD concentration also. As in the previous experiment, the total cell protein concentration decreased; the variation in cell carbohydrate concentration was smaller. Figure 29 shows the percent protein and carbohydrate of the sludge. There was a decrease in the percent protein, and an increase in the percent carbohydrate.

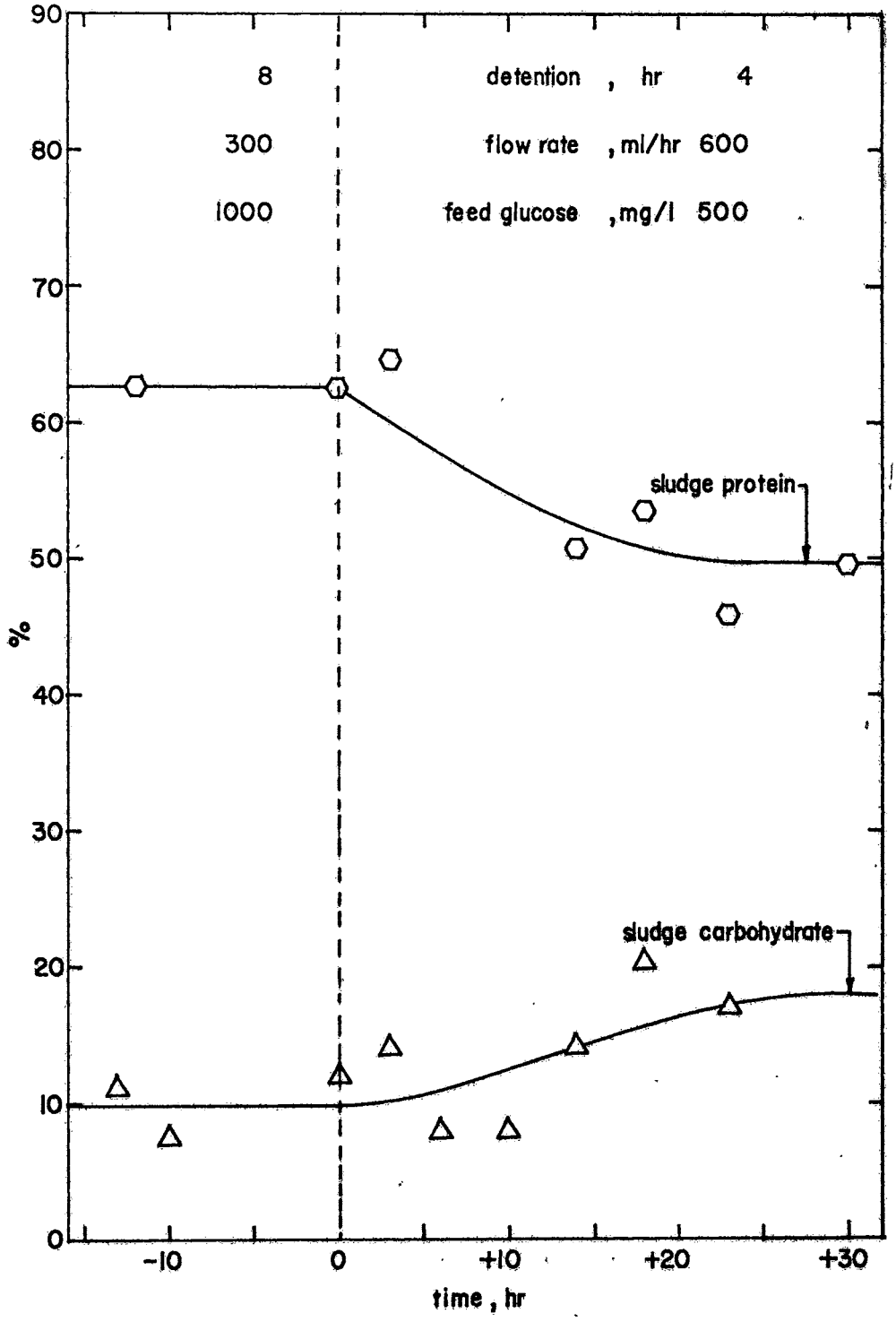

Figure 27 - Change in sludge composition of a system shock loaded from a dilution rate of 0.125 hour^{-1} to 0.25 hour^{-1} (constant loading).

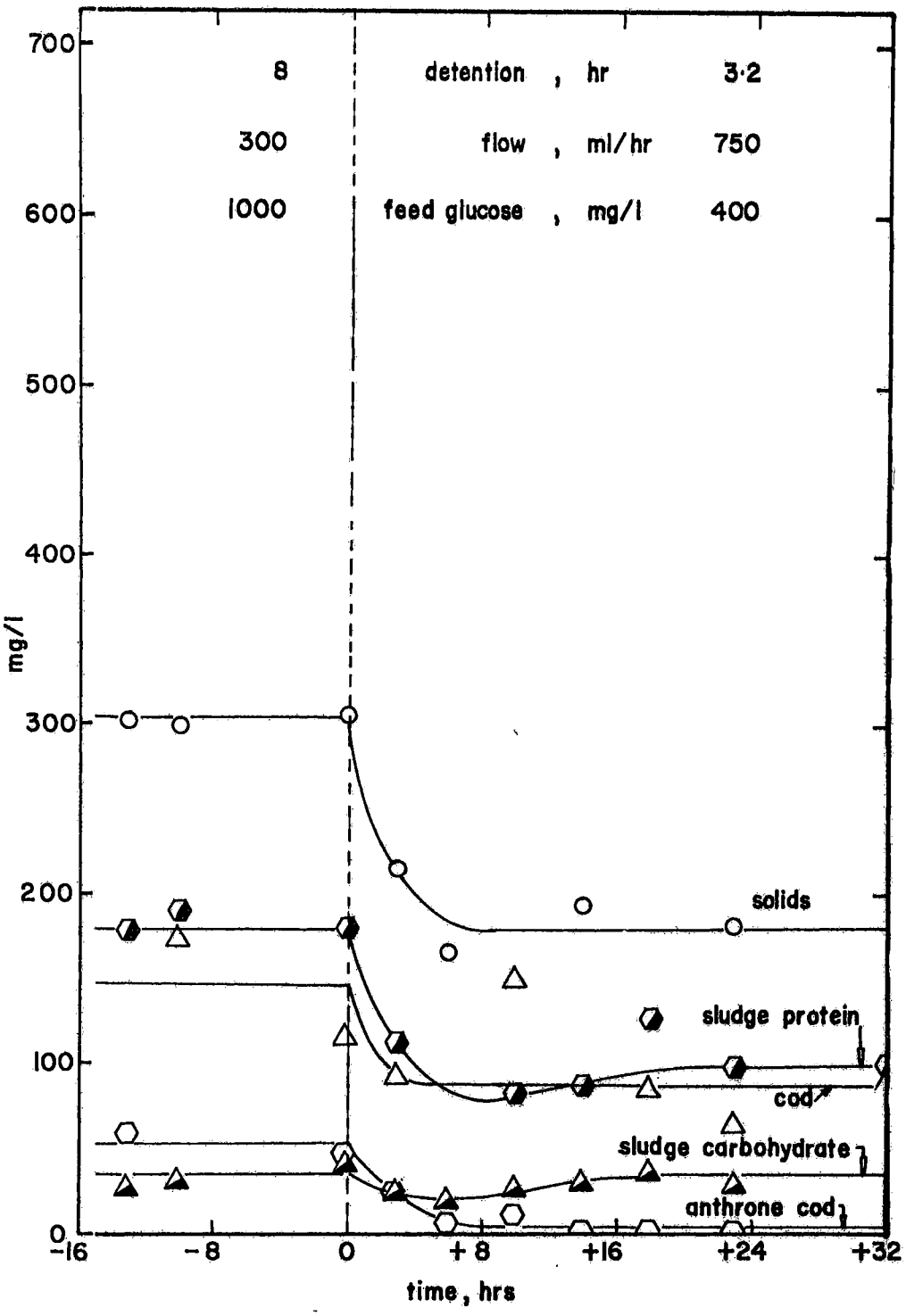

Figure 28 - Response of a system shock loaded from a dilution rate of 0.125 hour^{-1} to 0.313 hour^{-1} (constant loading).

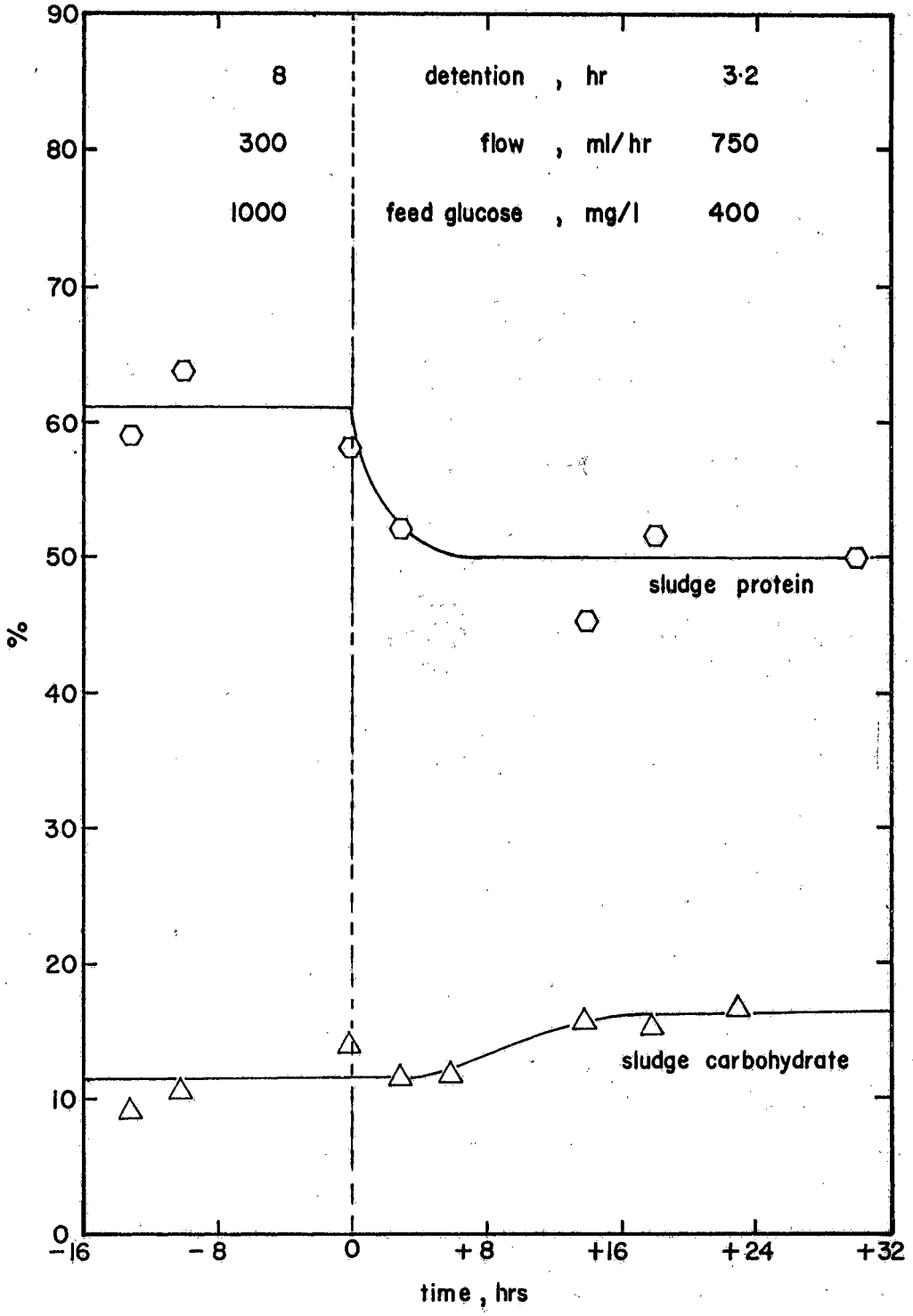

Figure 29 - Changes in sludge composition of a system shock loaded from a dilution rate of 0.125 hour^{-1} to 0.313 hour^{-1} (constant loading).

Figure 30 shows the response to the most severe shock load applied under constant loading conditions. The shock loading involved a change from a dilution rate of 0.125 $hour^{-1}$ (detention time eight hours) and 1000 mg/l glucose, to a dilution rate of 0.437 $hour^{-1}$ (detention time 2.29 hours) and feed concentration of 286 mg/l. The biological solids concentration decreased sharply from 350 mg/l to nearly 75 mg/l. The effluent COD dropped from 160 mg/l to approximately 80 mg/l. The anthrone COD decreased from 70 mg/l to nearly 10 mg/l.

Figure 31 summarizes the changes in yield and COD removal efficiency due to the hydraulic shock loadings. The changes (or differences) in yield and COD removal efficiency were obtained as the arithmetic difference in the respective values at steady states before and after the shock in each experiment. Comparing the yield values, it appears that the change in yield tends to decrease at dilution rates lower than the pre-shock value, and increase at higher dilution rates. However, the changes are more apparent under constant loading conditions than under constant concentration conditions. It is seen that at dilution rates in excess of 0.313 $hour^{-1}$, the change in yield decreases and approaches the pre-shock value under both conditions of operation, i.e., constant loading and constant concentration. Under both conditions of operation a hydraulic shock load resulting in a dilution rate of 0.25 $hour^{-1}$ or greater causes a significant decrease in COD

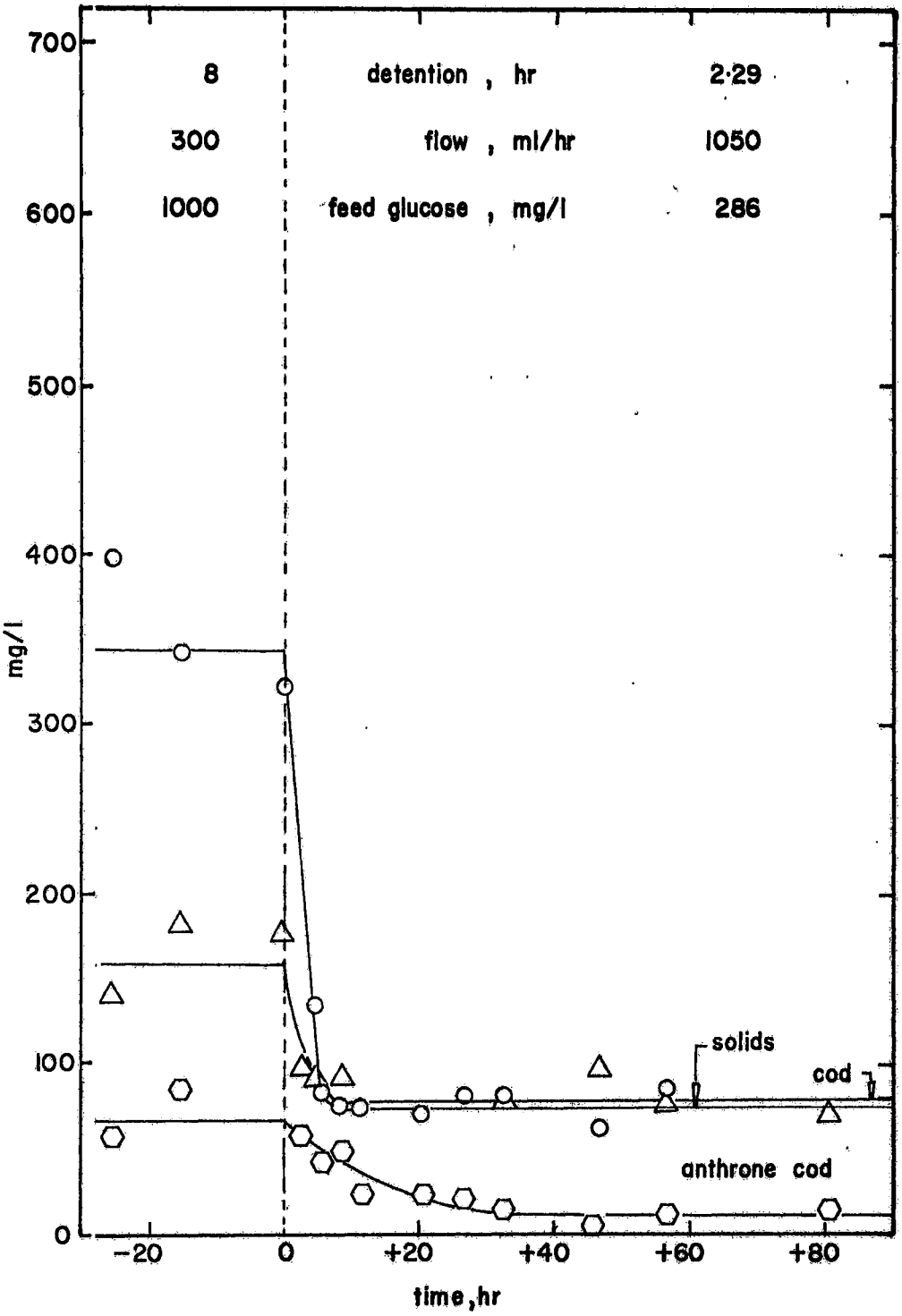

Figure 30 - Response of a system shock loaded from a dilution rate of 0.125 hour^{-1} to 0.437 hour^{-1} (constant loading).

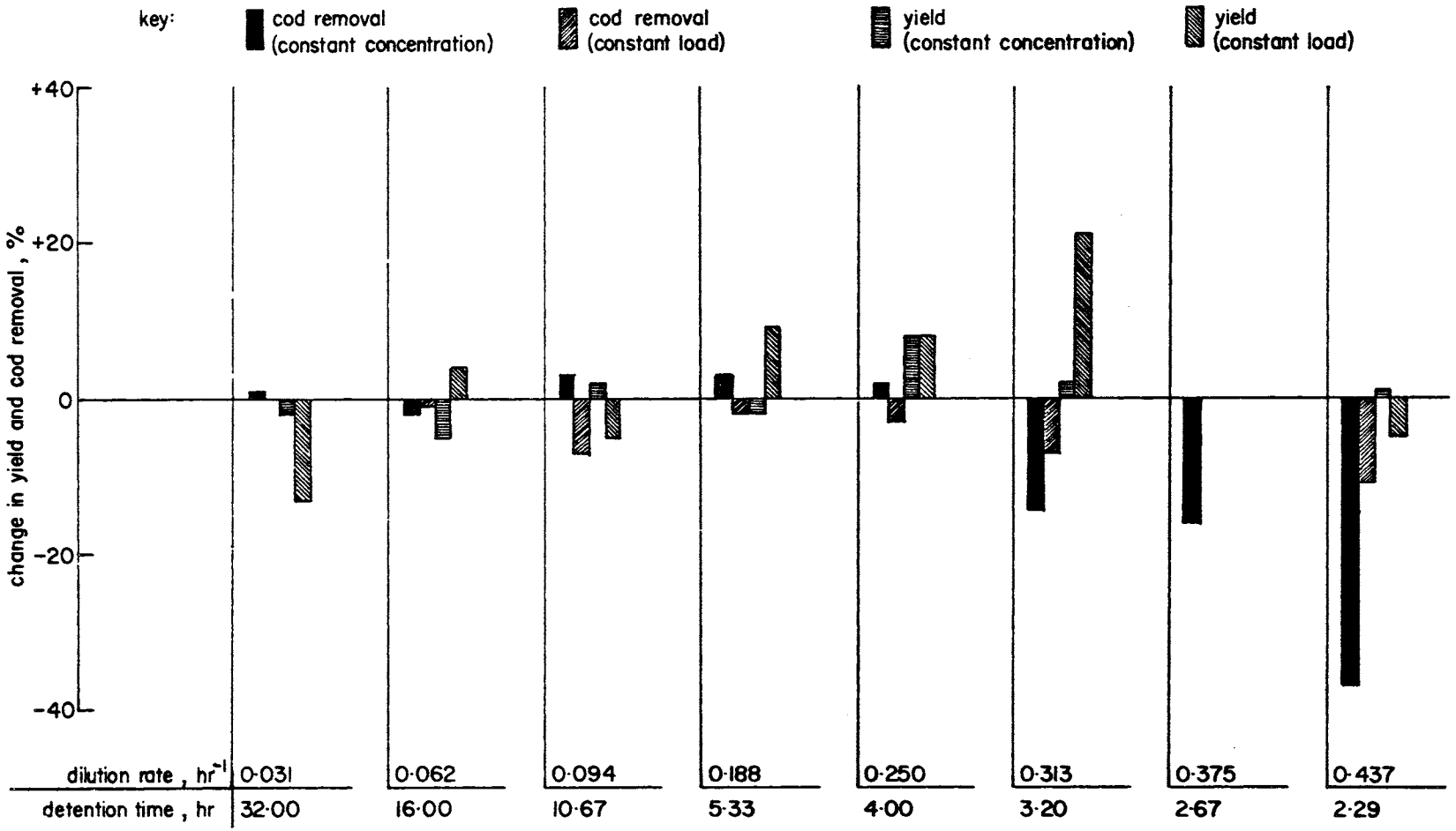

Figure 31 - Change in yield and COD removal efficiency between the initial and final steady states under hydraulic shock loading conditions.

removal efficiency. However, this decrease in efficiency is greater under constant concentration conditions than at constant loading conditions.

In Table III the effects of hydraulic shocks of the constant concentration type on effluent COD are summarized for all post-shock dilution rates. With regard to the transient state COD and the new steady state COD, the shock loads which resulted in an increase in detention time (from eight hours to thirty-two, sixteen, and 10.67 hours, respectively) did not cause an impairment of efficiency. On the other hand, the "overloading" cases which resulted in reactor detention times of 5.33, 4.00, 3.20, 2.67, and 2.29 hours, respectively, caused a systematic increase in transient state COD, new steady state COD, and maximum amount of COD due to intermediates; these parameters increased with the increase in flow rate after the shock. In all cases except the last (2.29 hours), the transient state COD was higher than both the old and the new steady state COD values. At the 2.29 hour detention time, the transient state was not clearly defined by a peak value, because the system was obviously approaching the region of cell wash-out. Discounting the extreme shock, the time to reach the transient state peak did not vary much for the several overloads.

Table IV shows the results for all experiments run under constant daily organic loading conditions. As the detention time was decreased from thirty-two hours to 2.29

TABLE III

CHANGES IN EFFLUENT COD IN RESPONSE TO HYDRAULIC SHOCK LOADS UNDER CONDITIONS OF CONSTANT SUBSTRATE CONCENTRATION

Initial Conditions: Detention Time, 8 hours; Dilution Rate, 0.125 $hour^{-1}$; Feed Glucose, 1000 mg/l

Final Detention Time	hours	32.00	16.00	10.67	5.33	4.00	3.20	2.67	2.29
Final Dilution Rate	$hour^{-1}$	0.031	0.062	0.094	0.188	0.25	0.313	0.375	0.437
Initial Steady State COD	mg/l	152	80	94	52	80	75	138	80
COD Removal Efficiency at Initial Steady State	%	86	92	91	95	92	93	87	92
Maximum (Minimum) Transient State COD	mg/l	90	112	68	62	142	306	360	480
COD Removal Efficiency	%	92	89	94	94	87	71	66	55
Time to reach the Above Transient	hour	37	18	18	4	3	3	4	15
Final Steady State COD	mg/l	80	112	68	24	64	228	312	480
COD Removal Efficiency	%	92	89	94	98	94	78	71	55
Time to reach the Above Steady State	hour	127	18	18	22	9	8	7	15
Maximum Intermediates	mg/l	56	68	78	28	110	282	348	406
Time to reach the Above Value	hour	75	18	0	6	3	3	4	15

TABLE IV

CHANGES IN EFFLUENT COD IN RESPONSE TO HYDRAULIC SHOCK LOADS UNDER CONDITIONS OF CONSTANT DAILY ORGANIC LOADING

Initial Conditions: Detention Time, 8 hours; Dilution Rate, 0.125 $hour^{-1}$; Feed Glucose, 1000 mg/l

Final Dilution Rate	$hour^{-1}$	0.031	0.062	0.094	0.188	0.25	0.313	0.437
Final Detention Time	hour	32	16	10.67	5.33	4.00	3.20	2.29
Feed Concentration After Shock	mg/l	4000	2000	1333	667	500	400	286
Initial Steady State COD	mg/l	112	70	52	52	124	148	160
COD Removal Efficiency	%	89	93	95	95	88	86	85
Maximum (Minimum) Transient State COD	mg/l	760	160	168	42	80	88	80
COD Removal Efficiency	%	82	92	88	94	85	79	74
Time to reach the Above COD	hour	86	23	39	16	10	18	10
Final Steady State COD	mg/l	424	160	168	50	80	88	80
Final COD Removal Efficiency	%	90	92	88	93	85	79	74
Time to reach the Above COD	hour	192	23	39	50	10	18	10
Maximum Intermediates	mg/l	272	112	68	28	72	100	68
Time to reach the Above Value	hour	86	23	39	50	14	8	30

hours in the several runs, the maximum value of the transient state COD decreased from 760 mg/l to 80 mg/l. Similarly, the new steady state COD ranged from 424 mg/l to 80 mg/l. These trends were almost parallel to those in the changes in the influent organic concentration (4000 mg/l to 286 mg/l as the detention time ranged from thirty-two to 2.29 hours). However, based on COD removal efficiencies, shock loads resulting in reactor detention times of greater than four hours did not indicate any serious damage. When the detention time after the shock was less than four hours, there was a clear disruption of COD removal efficiency. The amount of COD due to intermediates was a maximum at thirty-two hours detention time.

Table V shows the steady state parameters before and after the shock for all hydraulic shock loading experiments. Under constant concentration conditions, a shock load causing an increase in detention time tended to increase the protein content of the sludge and reduce the carbohydrate content, compared to the corresponding values before the shock. The reverse situation existed when the shock loading resulted in a decrease in detention time; i.e., an increase in flow rate caused a decrease in the protein content of the sludge with a simultaneous increase in the carbohydrate content. However, under constant loading conditions, any change in flow rate caused an increase in carbohydrate content and decrease in protein content of the sludge.

Table VI shows the yield of cells, protein, and carbo-

TABLE V

STEADY STATE PARAMETERS AT INITIAL AND FINAL STEADY STATES IN ALL HYDRAULIC SHOCK LOADING EXPERIMENTS

Detention Time, Hrs from to	Solids mg/l	COD mg/l	Anthrone COD mg/l	Protein %	Carbohydrate %	RNA %	DNA %	Feed Glucose mg/l
			Constant Concentration Conditions					
8.00	292	132	100	-	-	28	4.0	1000
32.00	276	80	60	-	-	36	2.5	"
8.00	510	80	32	50	20	-	-	"
16.00	444	112	44	57	15	-	-	"
8.00	460	94	16	37	12	-	-	"
10.67	500	68	12	43	11	-	-	"
8.00	580	52	38	58	15	-	-	"
5.33	580	24	20	54	16	-	-	"
8.00	436	80	40	57	21	-	-	"
4.00	548	64	20	48	23	-	-	"
8.00	532	74	34	52	20	-	-	"
3.20	468	228	16	41	26	-	-	"
8.00	536	138	22	55	19	-	-	"
2.67	436	312	10	48	21	-	-	"
8.00	540	80	12	49	12	18	-	"
2.29	294	480	76	46	14	25	-	"
			Constant Loading Conditions					
8.00	412	112	72	50	7	28	3.5	1000
32.00	1160	424	416	46	16	17	6.5	4000
8.00	420	72	8	48	6	-	-	1000
16.00	896	160	48	40	10	-	-	2000
8.00	564	52	26	62	8	-	-	1000
10.67	632	168	100	58	10	-	-	1333
8.00	536	52	24	-	-	-	-	1000
5.33	404	52	24	-	-	-	-	667
8.00	380	124	48	-	-	-	-	1000
4.00	220	80	8	50	18	-	-	500
8.00	304	148	54	61	12	-	-	1000
3.20	180	88	4	50	18	-	-	400
8.00	344	160	68	-	-	-	-	1000
2.29	76	80	12	-	-	-	-	286

TABLE VI

YIELD OF CELLS, PROTEIN AND CARBOHYDRATE AT INITIAL AND FINAL STEADY STATES IN ALL HYDRAULIC SHOCK LOADING EXPERIMENTS

Final (Shock)		Constant Concentration Conditions						Constant Loading Conditions					
Detention Time hr	Dilution Rate hr^{-1}	Percent Yield of						Percent Yield of					
		Solids		Protein		Carbohydrate		Solids		Protein		Carbohydrate	
		ISS*	FSS**	ISS	FSS	ISS	FSS	ISS	FSS	ISS	FSS	ISS	FSS
32.00	0.031	32	30	-	-	-	-	43	30	21	14	3.0	6.0
16.00	0.062	52	47	26	27	10.0	7.2	42	46	20	19	2.5	4.1
10.67	0.094	48	50	18	22	5.8	5.5	56	51	35	30	4.5	5.3
5.33	0.188	58	56	34	28	9.0	9.3	53	62	-	-	-	-
4.00	0.250	47	55	27	26	9.9	12.5	41	49	26	23	4.1	8.9
3.20	0.313	54	56	28	23	11	14.9	33	54	20	30	3.8	10.7
2.67	0.375	58	58	32	27	12	13.9	-	-	-	-	-	-
2.29	0.437	50	51	25	24	6.0	6.9	38	33	-	-	-	-

*Initial Steady State (Before Shock at 8-hour Detention Time)
**Final Steady State (After Shock at the Indicated Detention Time)

hydrate based on pre-shock and post-shock steady state values. Under conditions of constant concentration, the change in yield of cells from pre-shock to post-shock steady state is less than the variation generally seen with conditions of constant loading. A reduction in flow rate (dilution rate) causes an increase in the yield of protein and decrease in the yield of carbohydrates under constant concentration conditions. However, under constant loading conditions, the cells synthesize more carbohydrates and less protein, as seen by the increase in yield of the former and decrease in yield of the latter. An increase in flow rate under constant concentration conditions causes a decrease in the yield of protein and increase in the yield of carbohydrates.

B. pH Shock Loads

Phosphate buffer systems were used for controlling the reactor pH and the concentration of phosphorus in the synthetic waste was held constant for all experiments. Organic buffers could not be used because of the fact that they would add to the organic matter and COD. Use of other inorganic buffering agents, e.g., carbonate buffer for certain ranges of pH did not seem advisable because this would have caused changes in the inorganic constituents of the synthetic waste which might also exert an effect on the response of the system. Since the pK values of the phosphate buffer system are 2·1, 6·8, and 12·7, they are good buffers only in the pH ranges of approximately 1·1-3·1,

5·8-7·8, and 11·7-13·7, i.e., within the range of pK values ± 1 pH unit; for this reason no reactor pH was studied in the range 4-5. The following table gives the influent pH in relation to the reactor (effluent) pH for the several experiments:

TABLE VII

pH VALUES IN INFLUENT AND FINAL STEADY STATE REACTOR (EFFLUENT) FOR ALL pH SHOCK LOADING EXPERIMENTS

Figures	Influent pH	Reactor (Effluent) pH
52	2.75	2.70
49, 50, 51	3.65	3.00
47, 48	5.60	3.15
*45, 46	5.20	3.25
*43, 44	5.20	3.20
41, 42	6.60	3.50
40	6.95	5.75
38, 39	6.85	6.15
36, 37	8.00	7.25
*34, 35	9.35	7.65
32, 33	9.35	7.95

*With Cell Recycle.

The difference between the influent and reactor pH values is due to the depression of the pH values by microbial production of CO_2 and metabolic acid. Comparing this table with Table II, it can be seen that in cases where more acid salts were used in the phosphate buffer system, the reactor pH tended to be more acid, and where more alkaline

salts were used the reactor pH tended to be more alkaline. However, it is to be noted that the final steady state reactor pH is in most cases in one or other of the three buffering zones ($\pm$ 1 pH unit from the pK) of the phosphate buffer system.

1. Increase in pH

The general scheme for graphical presentation of the data is the same as for hydraulic shock loads. All experiments were conducted at a reactor detention time of eight hours and with 1000 mg/l glucose in the feed.

Figure 32 shows the biochemical response of a system shock loaded from pH 6.55 to 7.95. The biological solids concentration exhibited a decreasing tendency during the period of changing pH, but recovered to approximately the original value. It can be seen that this change in pH did not cause any appreciable change in total COD or anthrone COD in the effluent. Figure 33 shows changes in the percent composition of the sludge. There was some decrease in the protein content and DNA content, which was accompanied by an increase in the carbohydrate content.

Figure 34 shows the result of an experiment over the same pH range, but in which recycle of solids was employed. In this case the reactor pH reached an average value of 7.65 at the new steady state after the shock. Under this condition the shock resulted in an increase in solids from 1100 mg/l to approximately 1500 mg/l. There was absolutely no change in effluent COD and anthrone COD, both of which

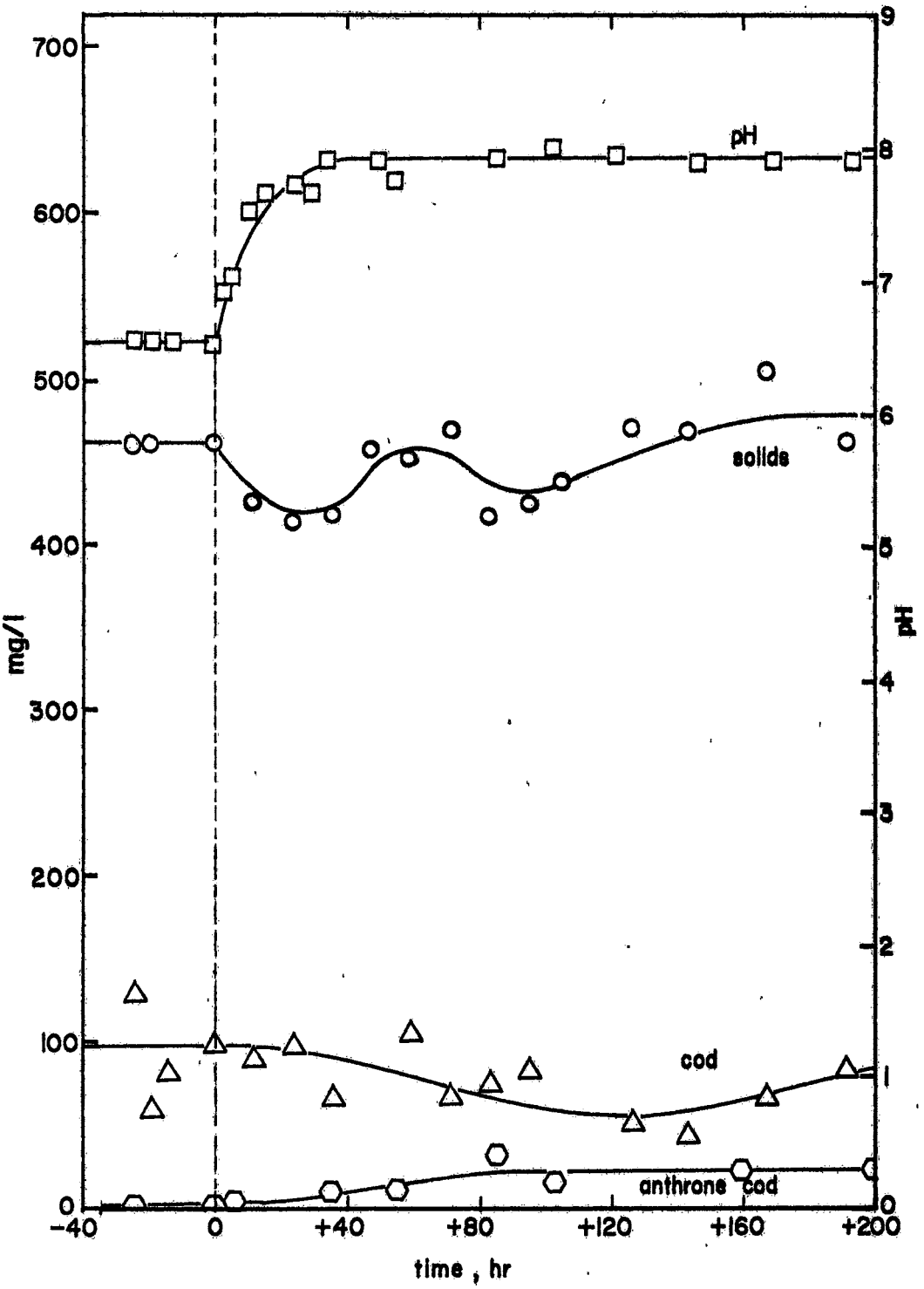

Figure 32 - Response of a system shock loaded from pH 6.55 to 7.95.

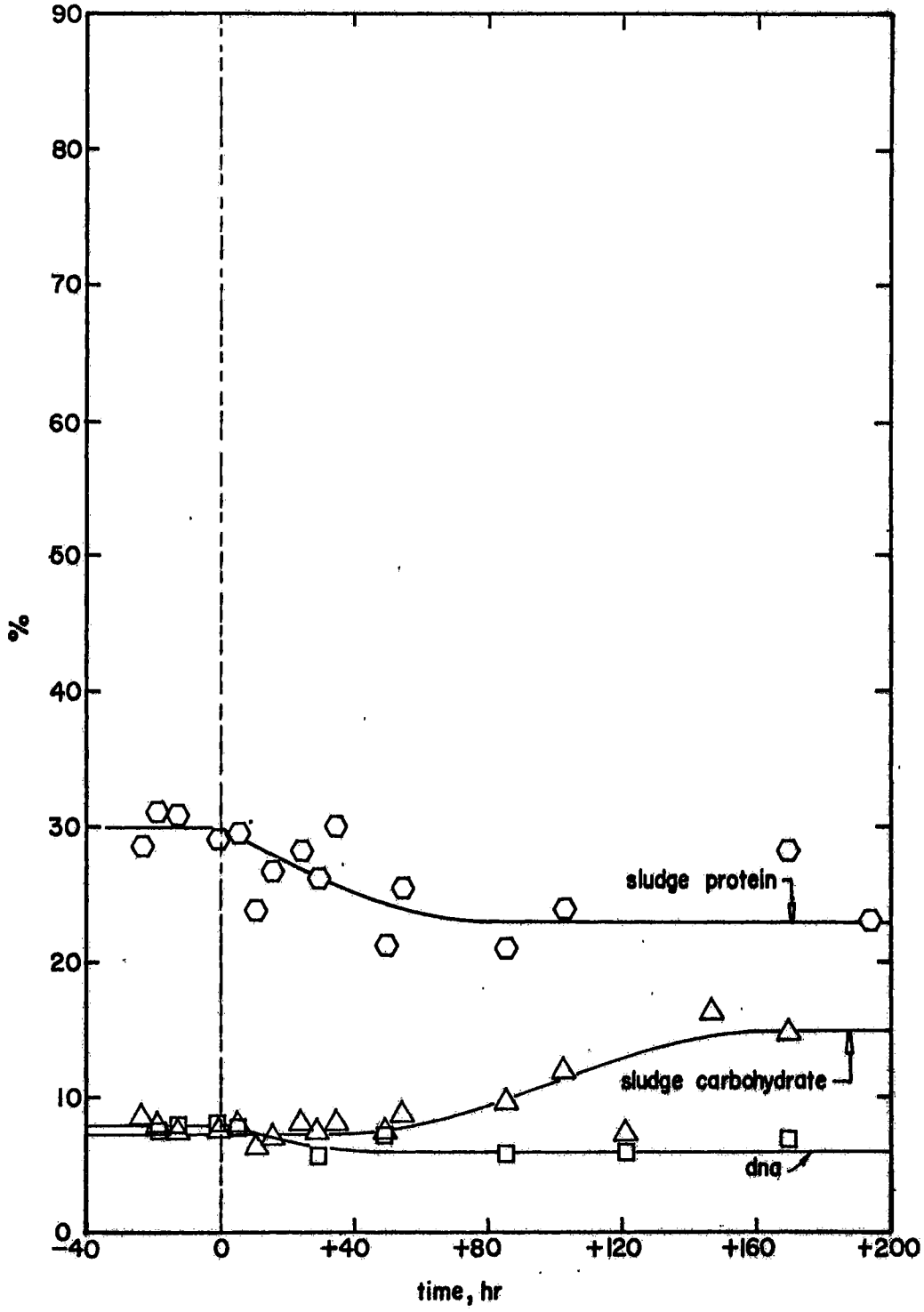

Figure 33 - Change in sludge composition of a system shock loaded from pH 6.55 to 7.95.

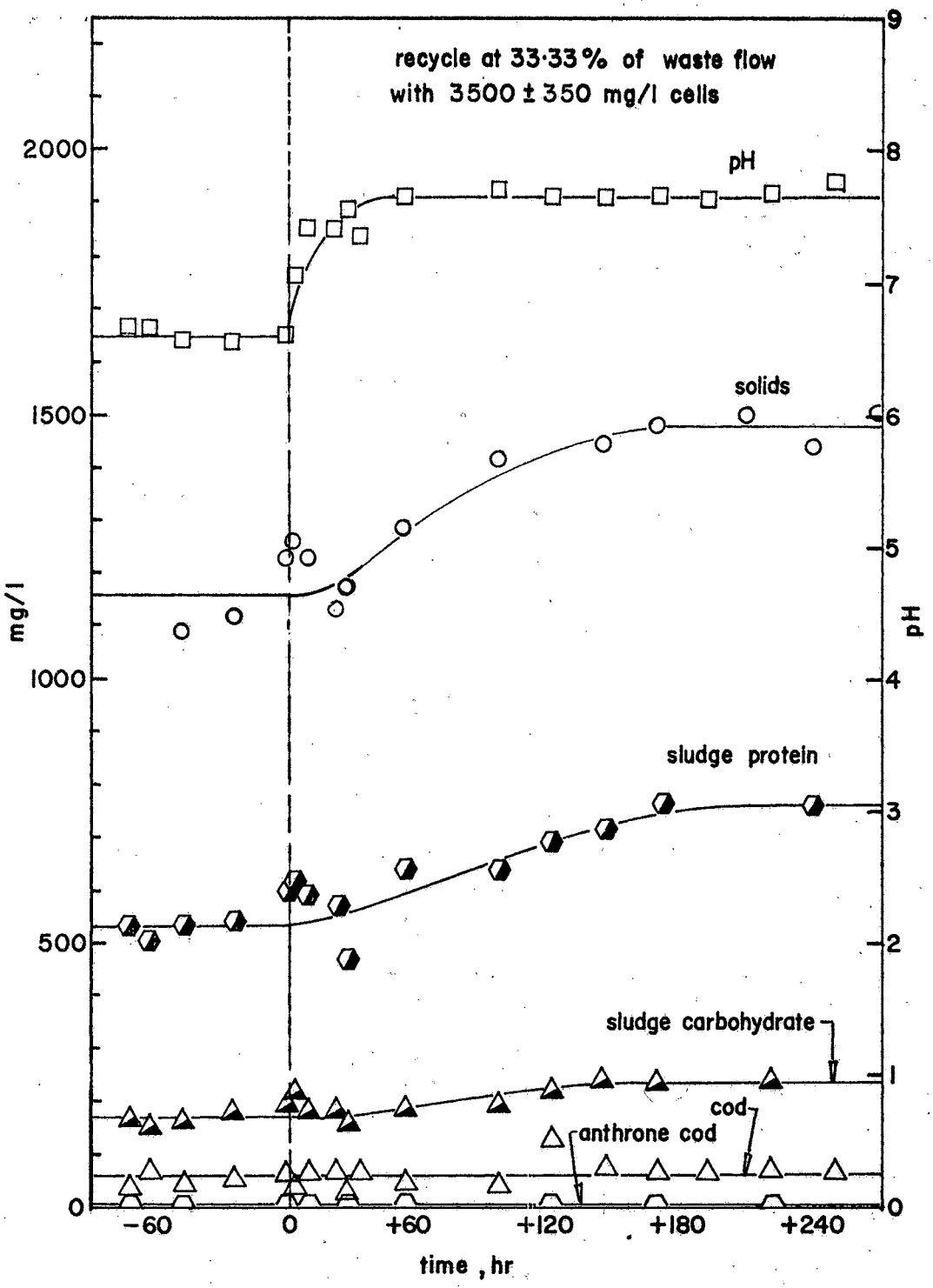

Figure 34 - Response of a system shock loaded from pH 6.60 to 7.65 (with recycle of sludge).

remained at very low levels. There was a significant increase in the total protein, and a slight increase in the total carbohydrate. Figure 35 shows the change in sludge composition. In contrast to the system without recycle, there was an increase in percent protein, but very little change in carbohydrate and DNA.

Figure 36 shows the biochemical response of a system which was shock loaded from pH 6.65 to 7.25. This is a somewhat milder shock on the alkaline side, compared to the previous one. The biological solids concentration fluctuated, but no upward or downward trend was noted. Very little change was noted in the filtrate COD and anthrone COD. In short, this shock load was easily assimilated. Figure 37 shows changes in sludge composition for the experiment. The percent protein dropped somewhat, as did the RNA and DNA. However, the carbohydrate content increased slightly.

2. Decrease in pH

The succeeding studies involved a change in pH toward the acid range. The response to a change from pH 6.65 to 6.15 is shown in Figure 38. It is seen that the solids gradually decreased from 480 mg/l to a transient low value of nearly 285 mg/l, and later rose to approximately 680 mg/l. At the lowest point of solids concentration there was a significant leakage of COD from the aerator. The anthrone COD rose only from 40 mg/l to 60 mg/l during this period. Figure 39 shows the changes in sludge composition

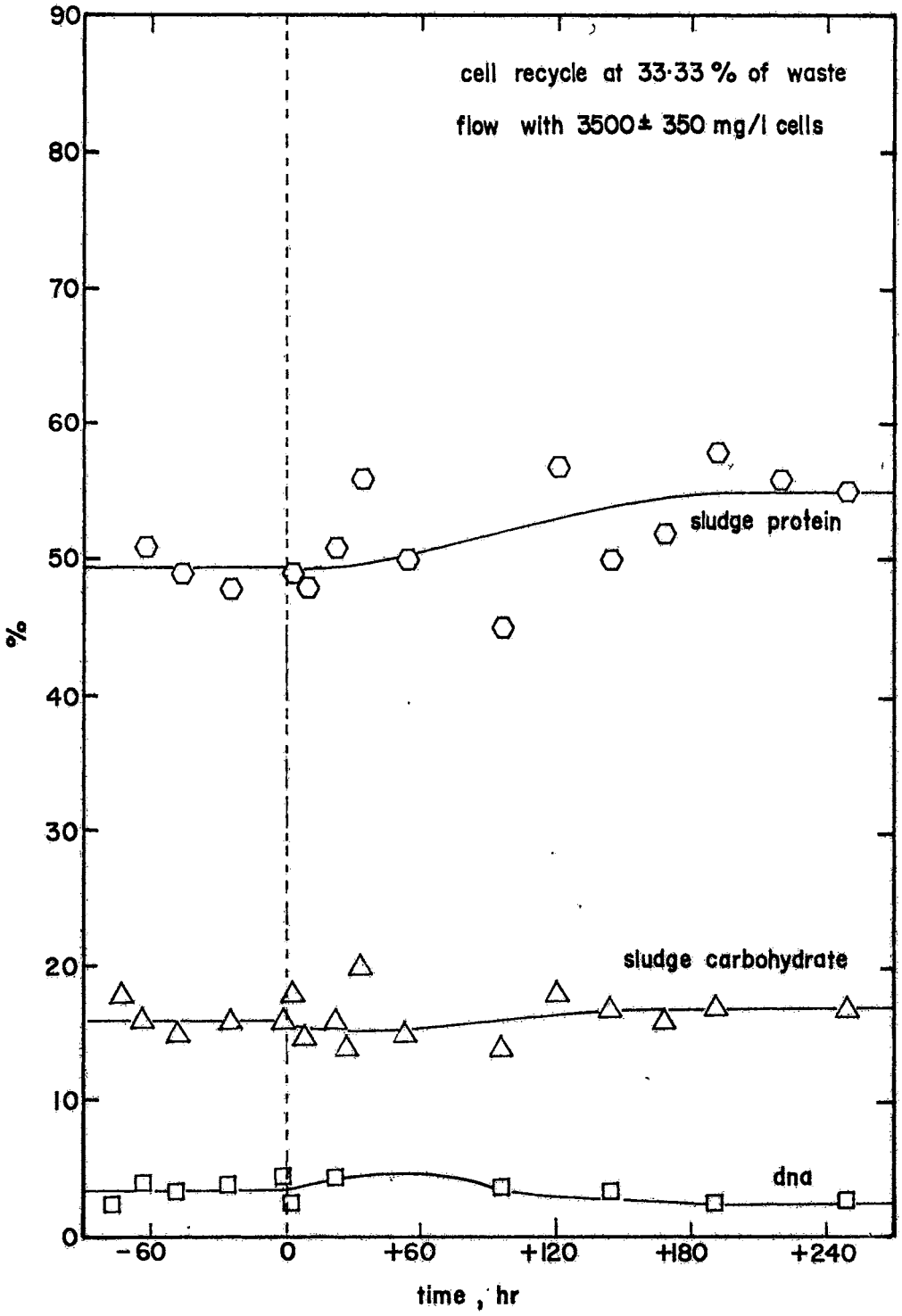

Figure 35 - Changes in sludge composition of a system shock loaded from pH 6.60 to 7.65 (with sludge recycle).

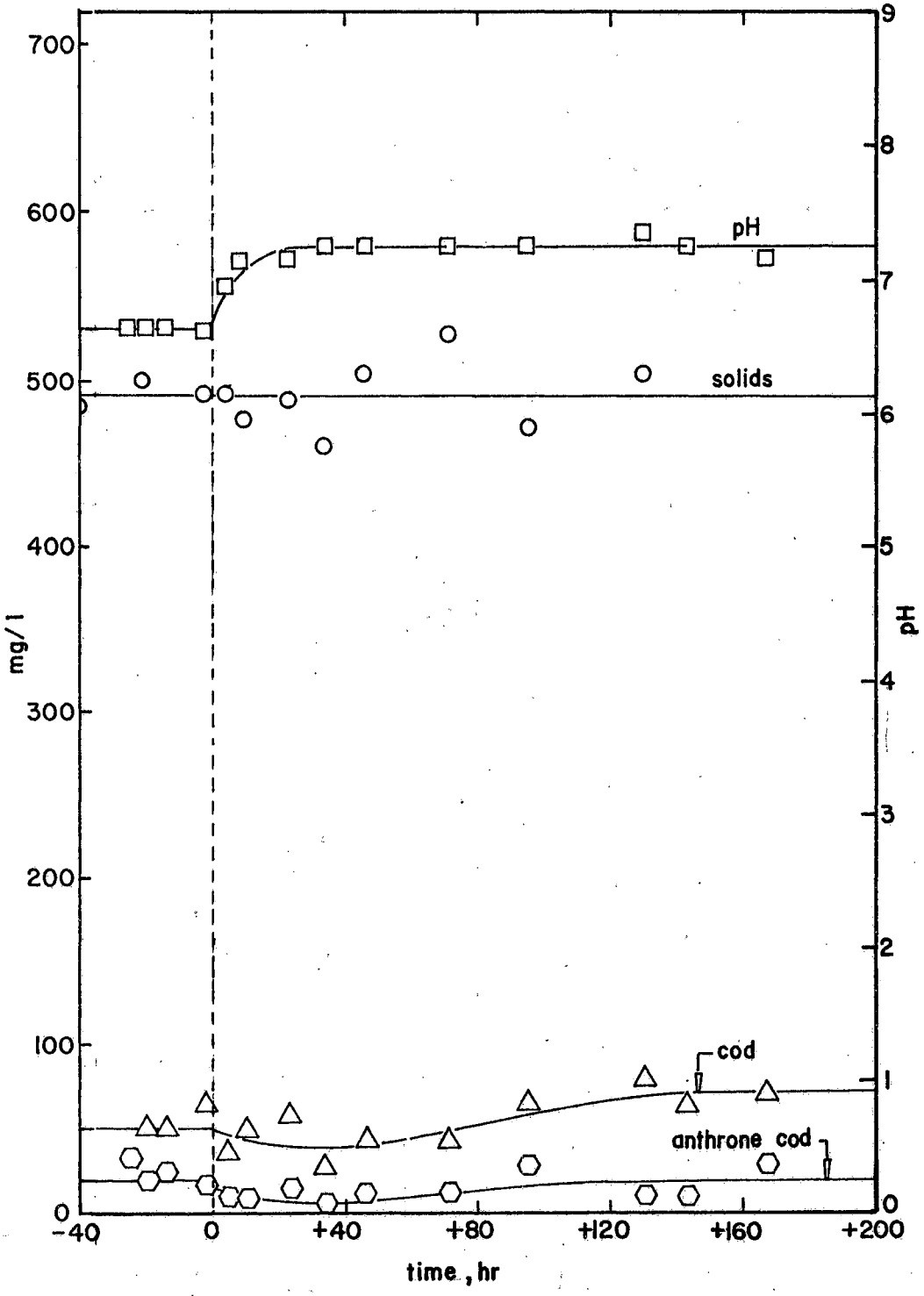

Figure 36 - Response of a system shock loaded from pH 6.65 to 7.25.

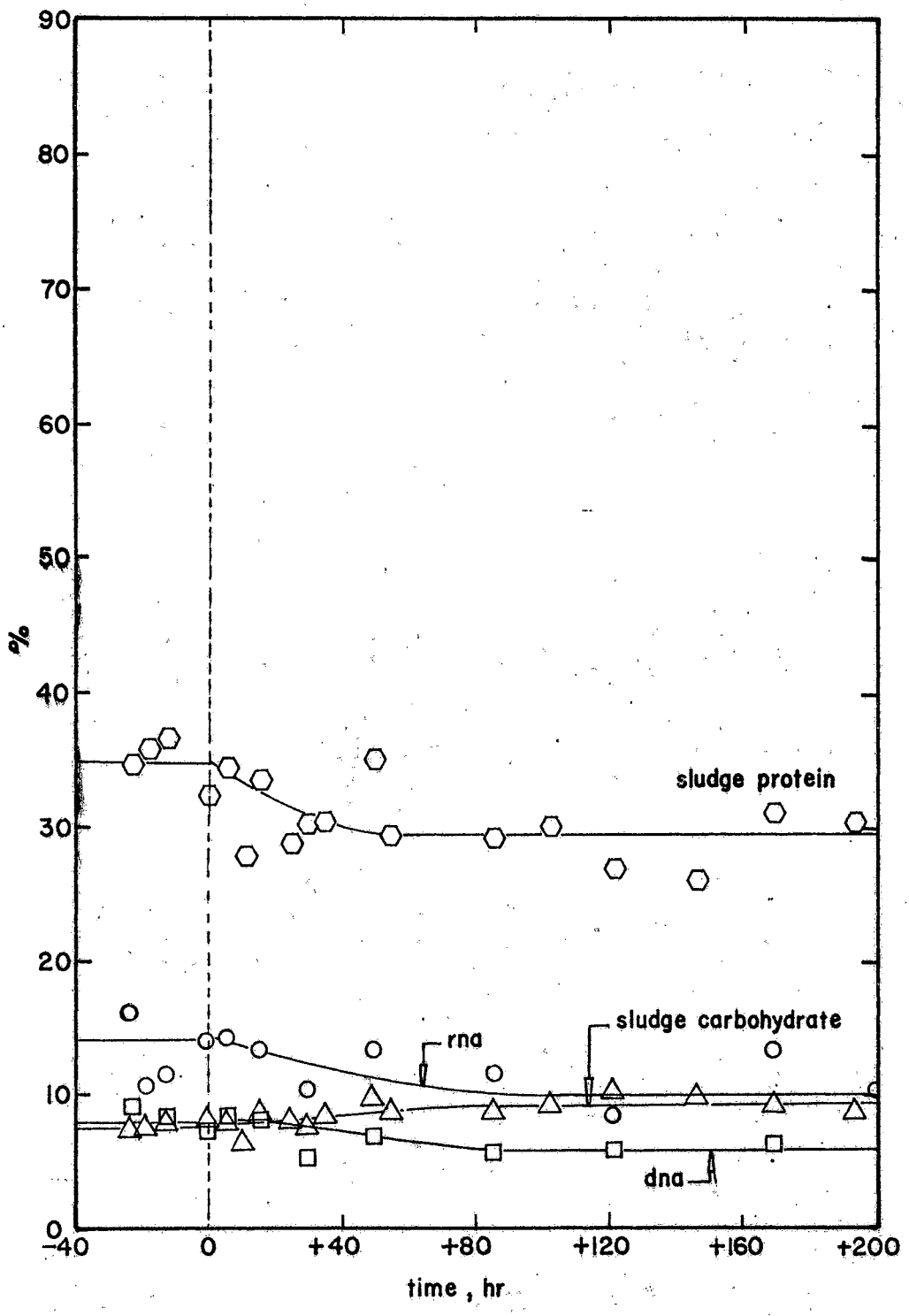

Figure 37 - Changes in sludge composition of a system shock loaded from pH 6.65 to 7.25.

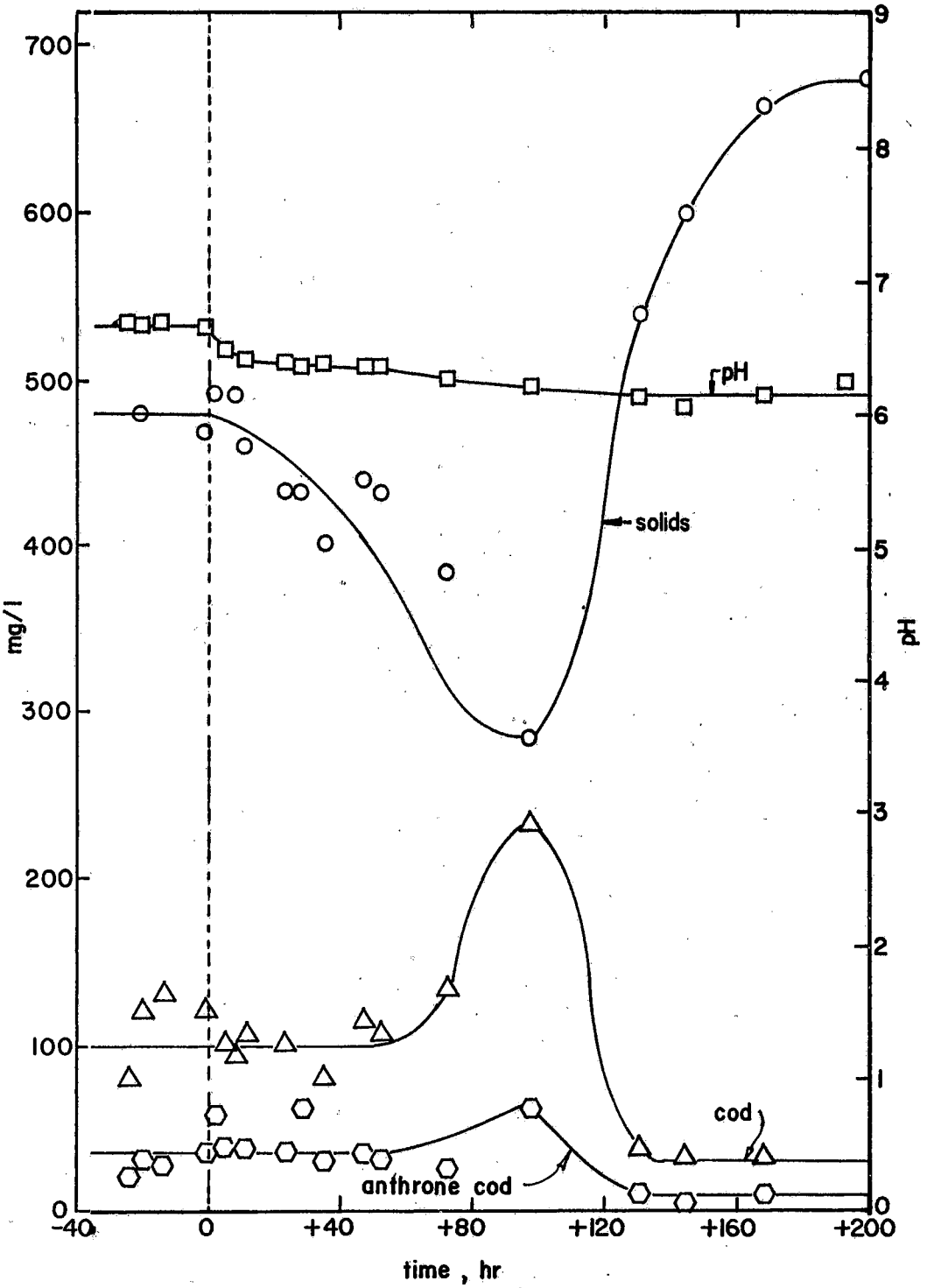

Figure 38 - Response of a system shock loaded from pH 6.65 to 6.15.

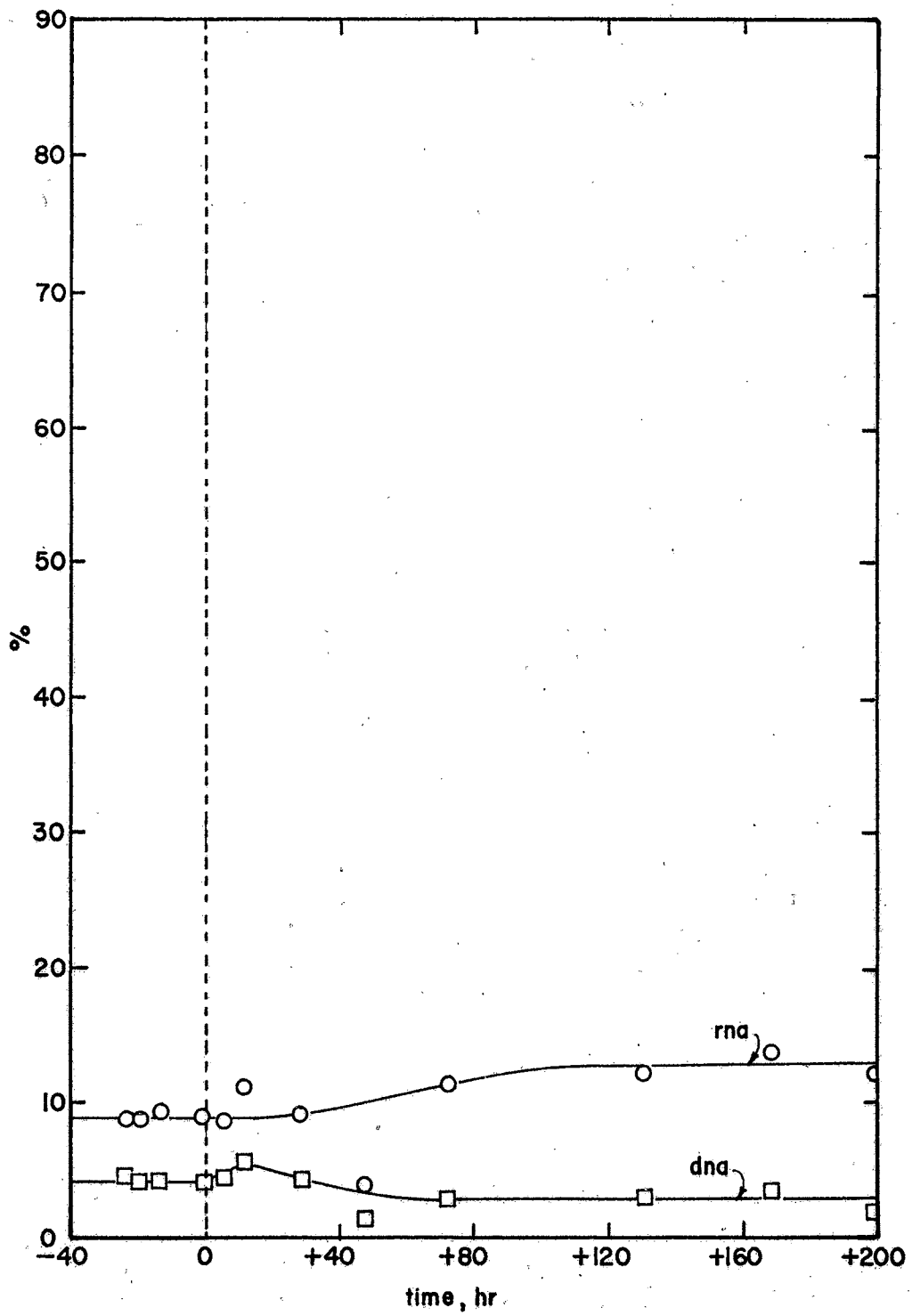

Figure 39 - Changes in sludge composition of a system shock loaded from pH 6.65 to 6.15.

during this experiment. There was some increase in the RNA content, and decrease in the DNA content.

Figure 40 shows the response of a system shocked from pH 6.70 to 5.75. Even though the drop in pH was greater in this experiment than in the previous one, the system response was less deleterious. It is seen that a significant decrease in biological solids concentration ensued as a result of the shock, but the COD removal efficiency was not decreased; it actually improved. It is interesting to note that, as in the previous experiment, acid conditions caused an increase in solids level at the new steady state.

Figure 41 shows the results for a more severe shock load with reactor pH reaching 3.50. During the transient state the biological solids dropped from 375 mg/l to nearly 65 mg/l, and then rose rapidly to a new level of approximately 600 mg/l. The COD of the effluent rose from 225 mg/l to 725 mg/l in approximately four detention periods (32 hours), and then declined until it reached a new steady state level of 30 mg/l. The total response was completed in eighteen detention periods (144 hours) from the point of shock. It is seen that while the system could eventually accommodate the new pH, the transition period was long and caused severe disruption of purification efficiency. The total protein and total carbohydrate concentration of the sludge dropped to values less than 50 mg/l, and then recovered. However, the protein concentration did not regain the value at the steady state prior to the shock.

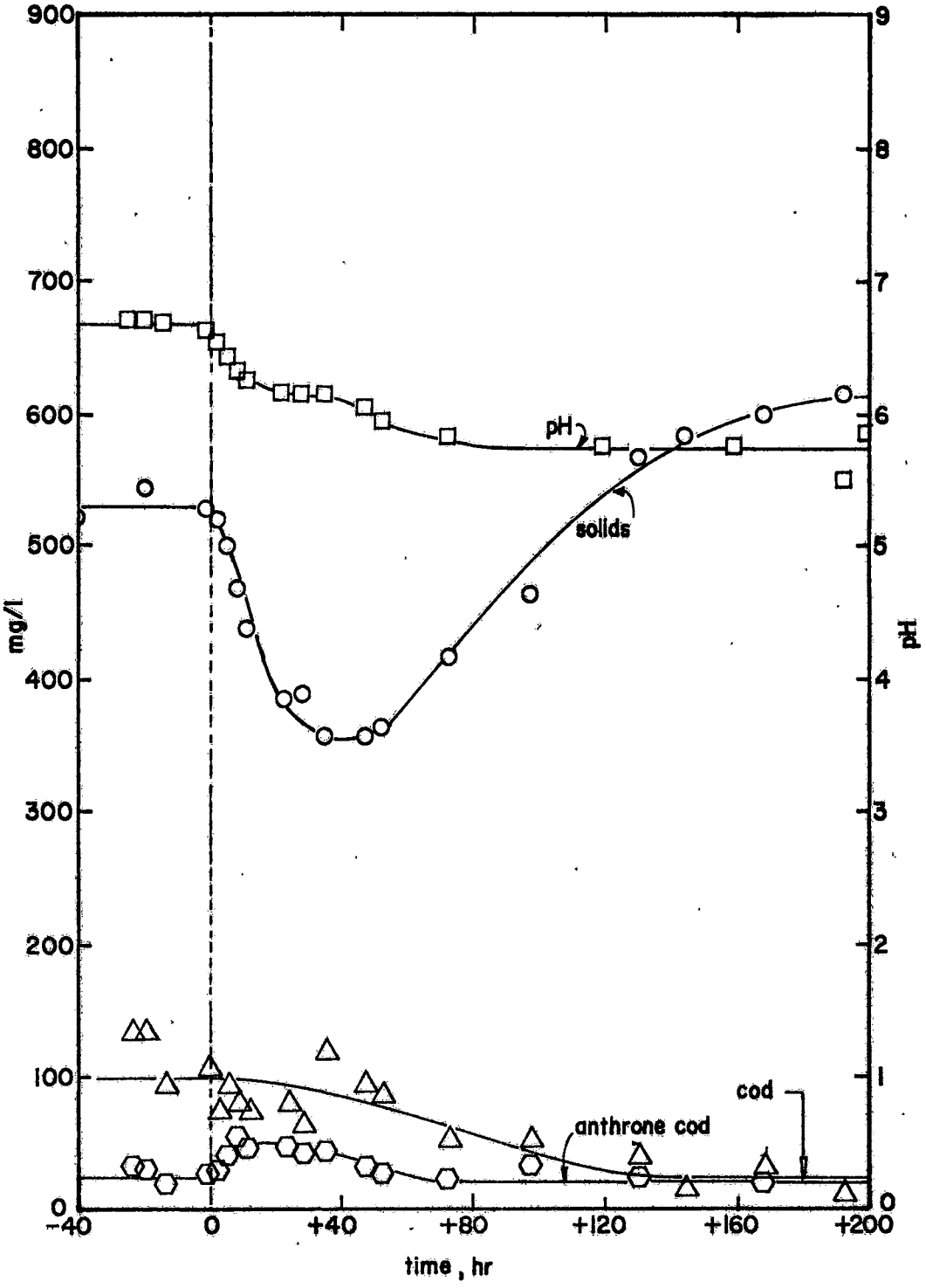

Figure 40 - Response of a system shock loaded from pH 6.70 to 5.75.

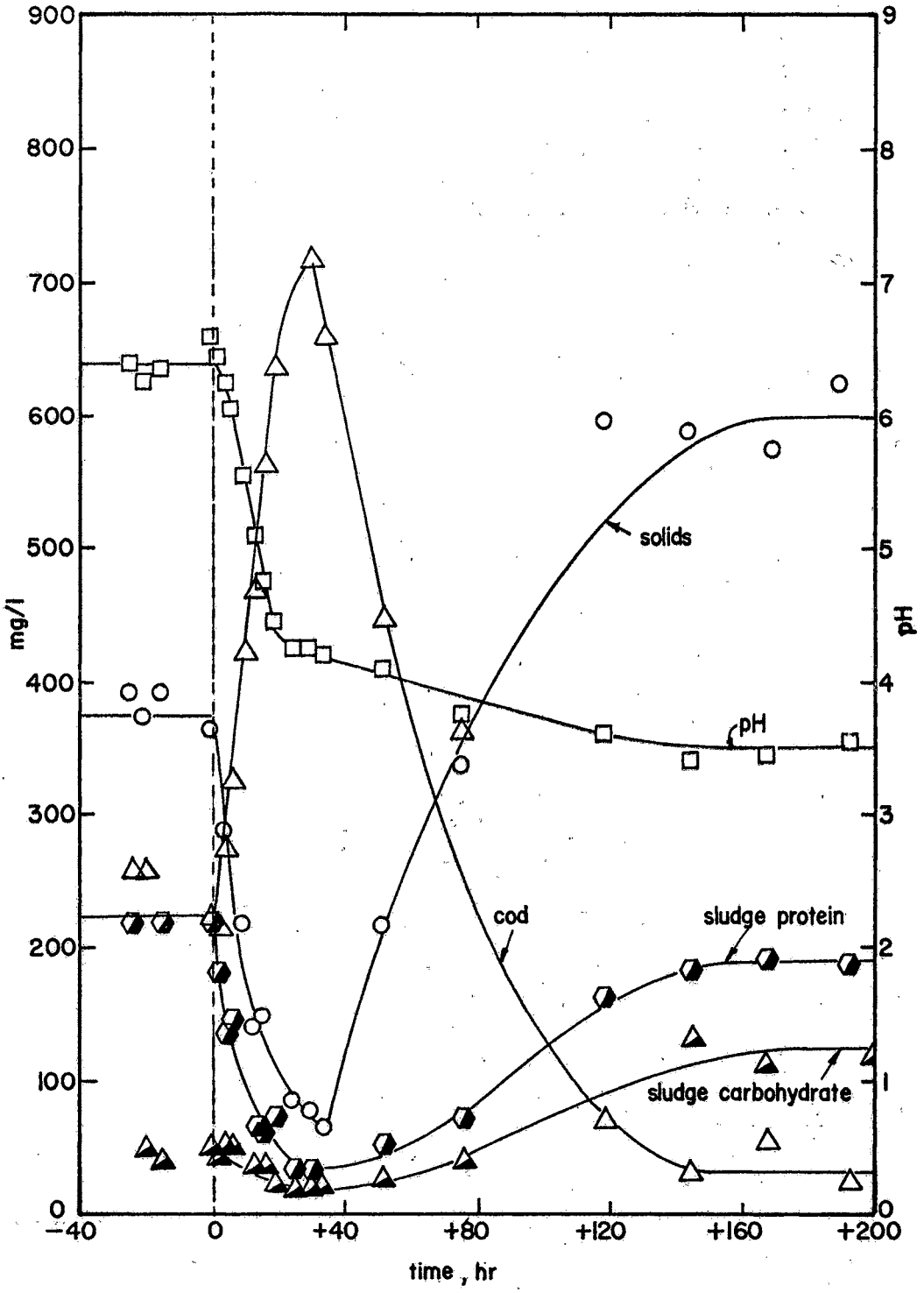

Figure 41 - Response of a system shock loaded from pH 6.40 to 3.50.

Figure 42 shows how much the sludge components can vary in response to such a severe change in reactor pH. The protein content dropped tremendously from 56 per cent to a transient low value of 22 per cent in approximately ten detention times, and then reached a new steady state value of 29 per cent. On the other hand, the carbohydrate content increased from 15 per cent to a transient peak value of 38 per cent at four detention periods, and then gradually declined to a new steady state value of 25 per cent. There was a reduction in the DNA content, also. Conspicuous changes in microbial predominance were noticed due to this severe change in pH. Details of the change will be presented below.

Figure 43 shows the response when the pH was changed from 6.65 to 3.20 in a system in which a high solids concentration was maintained by recycle of a major portion of the settled sludge. It is seen that the effluent COD remained at less than 150 mg/l at all times, even though the reactor solids level dropped from approximately 2240 mg/l to nearly 900 mg/l. The shock loading prevented maintenance of a high level of solids because some sludge was lost from the settling tank because of poor settleability. Only settled sludge could be recycled back to the reactor, and this caused a drop in reactor solids. Moreover, this shock load resulted in a less severe change in microbial predominance. The major portion of the sludge was composed of rod-shaped organisms, even though a minor portion consisted of filamen-

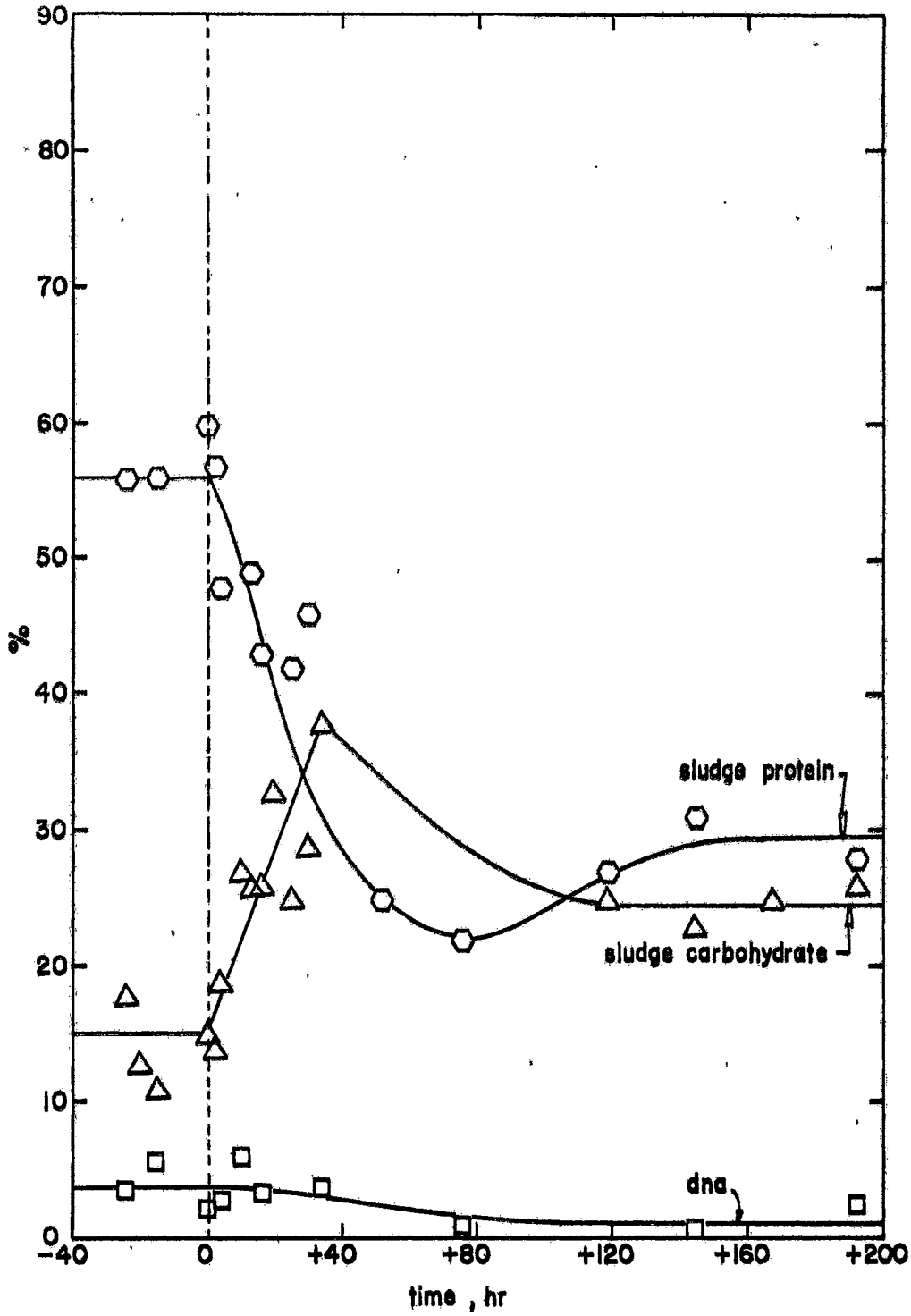

Figure 42 - Changes in sludge composition of a system shock loaded from pH 6.40 to 3.50.

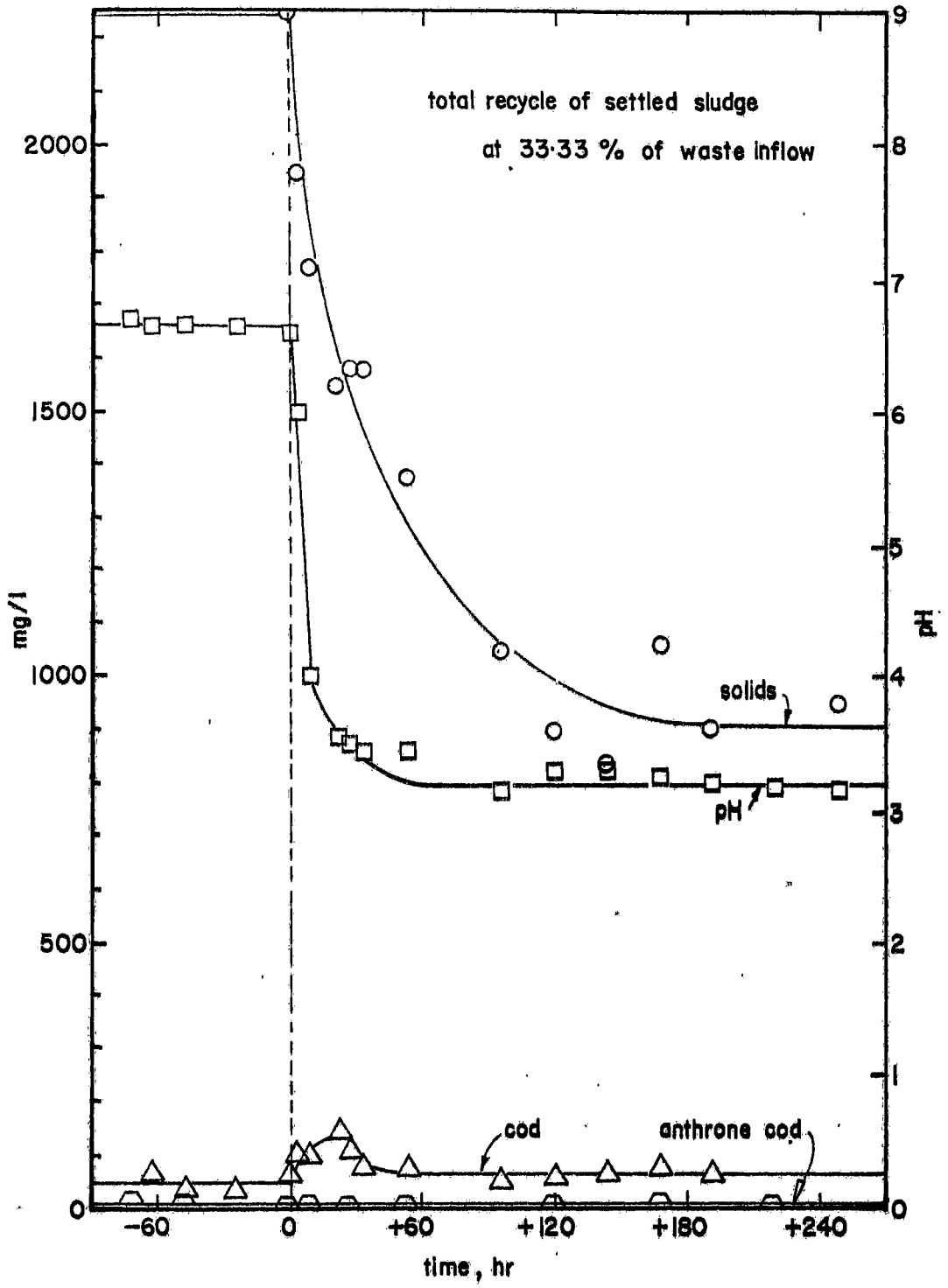

Figure 43 - Response of a system shock loaded from pH 6.65 to 3.20 (with total recycle of settled sludge).

tous types. This is probably one reason why the synthetic activity of the sludge was low during the course of the experiment, because from the previous two experiments it was seen that an acidic medium favored the growth of filamentous types rather than rods. Figure 44, showing the sludge composition, indicates that the decrease in percent protein content was less severe than in the previous two experiments; it decreased from 52 to 39 per cent. The carbohydrate content increased from 15 per cent to 36 per cent within a span of eighteen detention periods, and then dropped to 22 per cent. The percent DNA content also decreased.

Almost the same change in pH was imposed again under different recycle conditions; the pH change was from 6.45 to 3.25. The recycle sludge had an average concentration of 1000 mg/l and was pumped at 33 1/3 per cent of the rate of flow of the feed. This resulted in a reactor solids concentration of approximately 625 mg/l prior to the shock. The system response, which is shown in Figure 45, was intermediate between the responses of those shown in Figures 43 and 47, i.e., the one with total recycle and the other without cell recycle. The solids dropped to approximately 140 mg/l in seven detention periods, and then climbed to nearly 760 mg/l, a value higher than that maintained before the shock. The effluent COD reached a maximum value of approximately 600 mg/l compared to 900 mg/l for the flow through system (Figure 47). The major portion of the COD

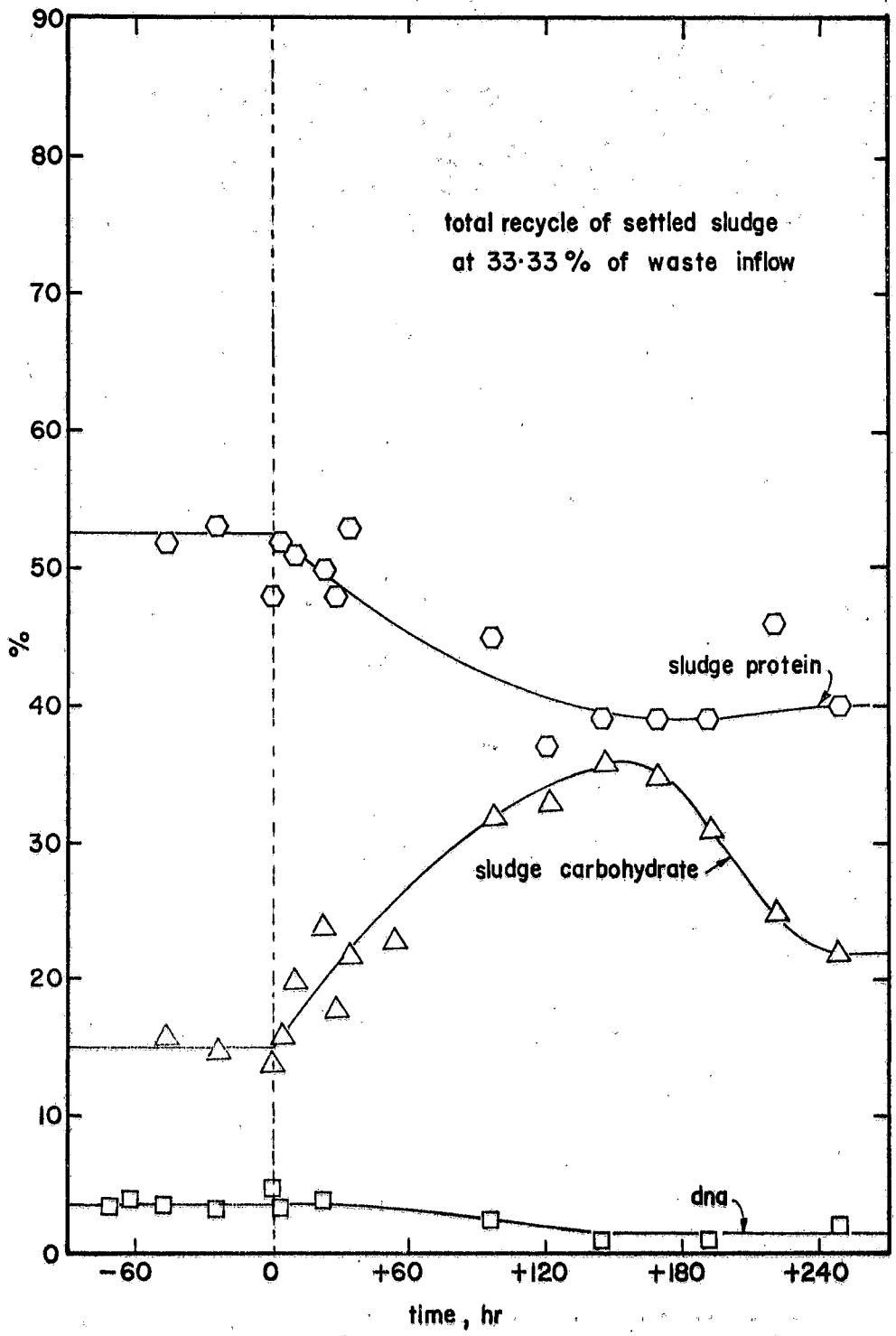

Figure 44 - Changes in sludge composition of a system shock loaded from pH 6.65 to 3.20 (with total recycle of settled sludge).

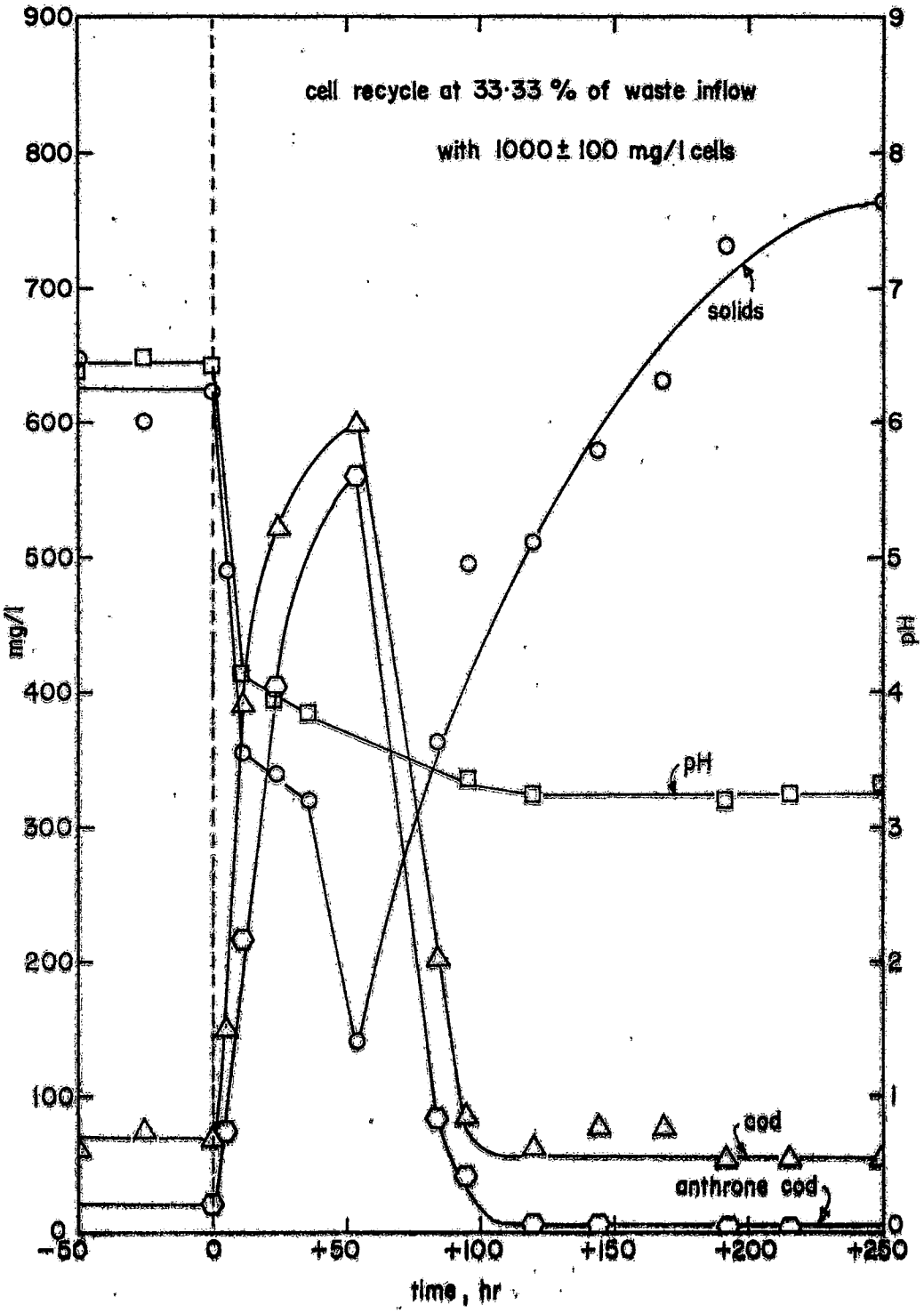

Figure 45 - Response of a system shock loaded from pH 6.45 to 3.25 (with partial recycle of sludge).

in the filtrate was contributed by carbohydrates, as shown by the close agreement between the total COD and the anthrone COD. Figure 46 shows changes in the percentage protein and carbohydrate of the sludge during this experiment. As for the flow through system (Figure 48), the protein content of the sludge decreased; in this case from 53 per cent to nearly 13 per cent, and then increased very slowly. On the other hand, the carbohydrate content rose from approximately 13 per cent to nearly 40 per cent in twelve detention times, and then decreased to 28 per cent.

Figure 47 shows the biochemical response of a system (no cell recycle) which was shock loaded from pH 6.60 to 3.15, approximately the same as in the previous experiments with cell recycle. The pH curve indicated a two-stage drop, first from 6.60 to 4.10 during the downward trend of the biological solids, and then to 3.15, apparently due to the upward trend of the solids concentration as the sludge acclimated to the new environment. The solids concentration dropped from 430 mg/l to nearly zero in four detention periods, and then some species became acclimated and began to grow to a new steady state solids level of approximately 730 mg/l. The filtrate COD rose from 66 mg/l to nearly 900 mg/l in the same time, and then gradually reduced to an average value of 120 mg/l. The anthrone COD curve paralleled the COD curve. Figure 48 shows changes in sludge composition during the experiment. The protein content decreased from nearly 51 per cent to approximately 21 per cent, and

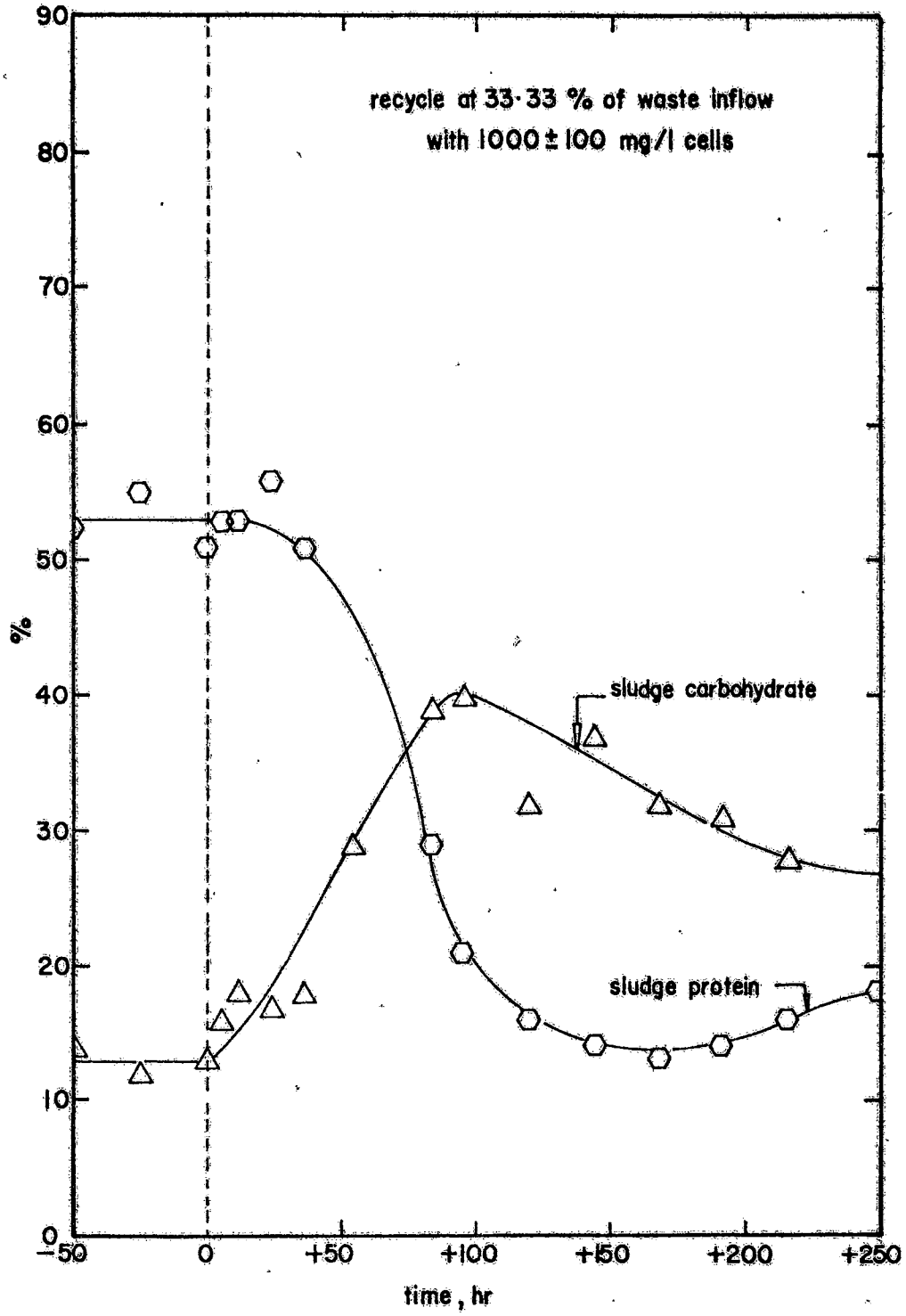

Figure 46 · Changes in sludge composition of a system shock loaded from pH 6.45 to 3.25 (with partial recycle of sludge).

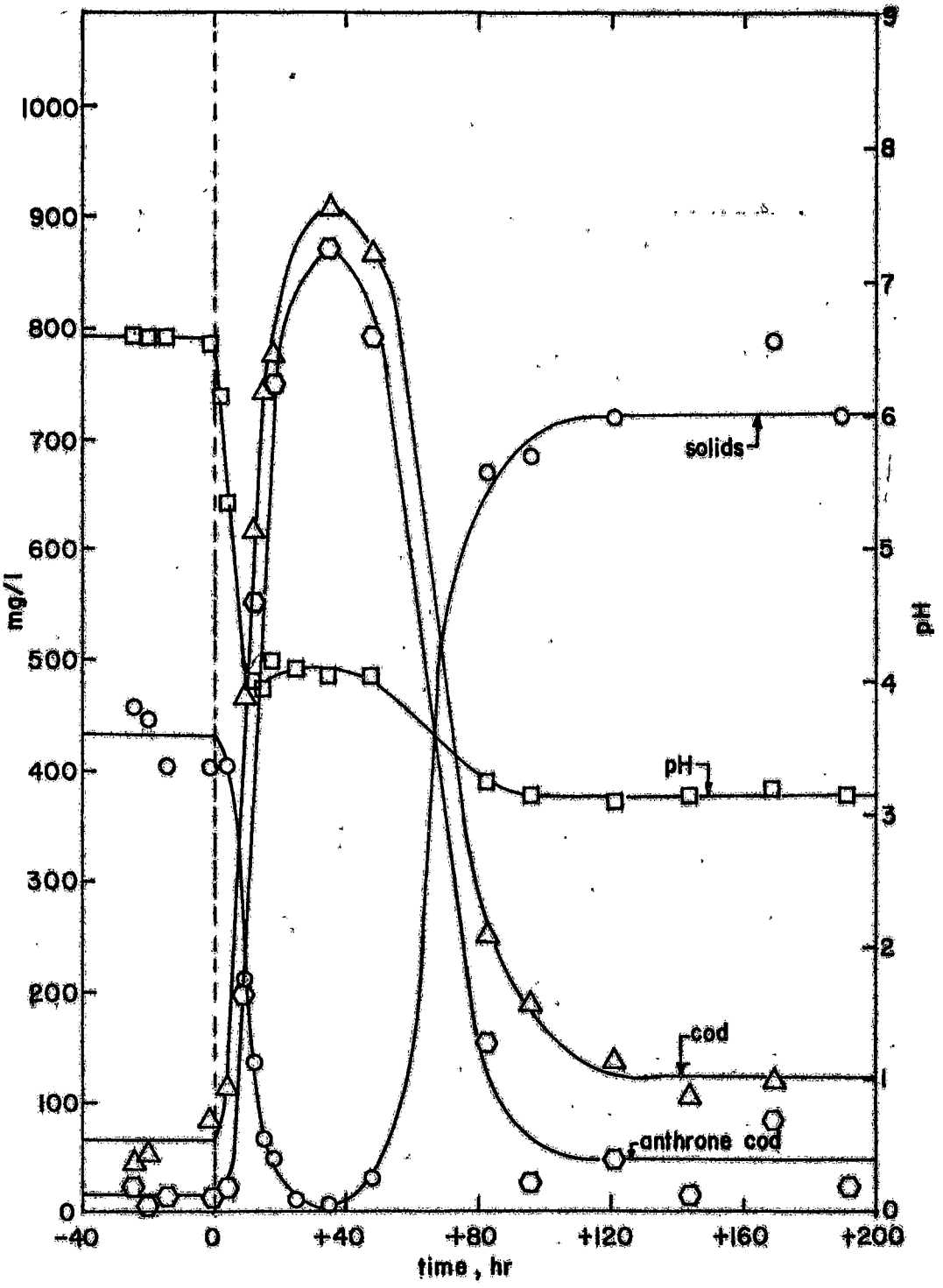

Figure 47 - Response of a system shock loaded from pH 6.60 to 3.15.

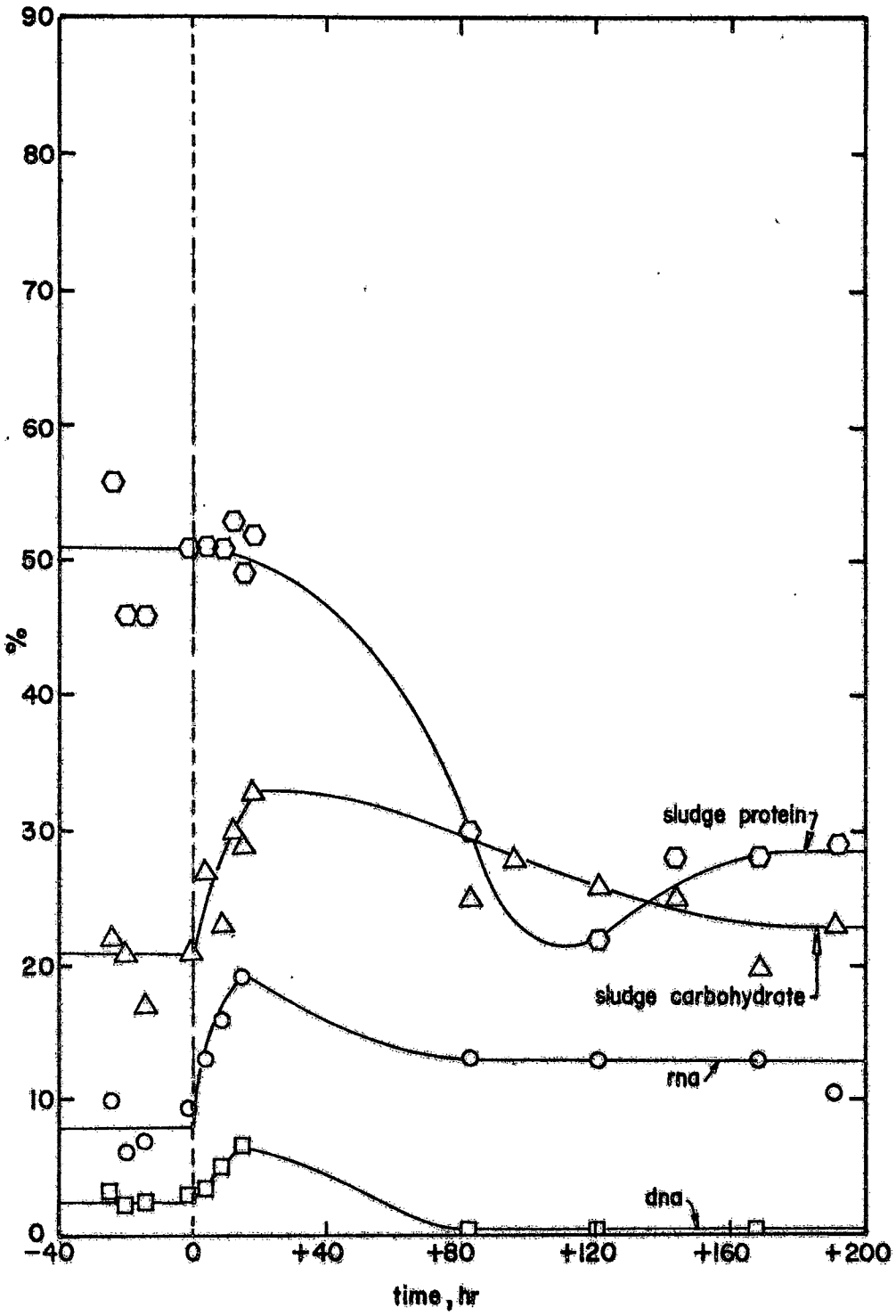

Figure 48 Changes in sludge composition of a system shock loaded from pH 6.60 to 5.15.

then rose to 28 per cent. In the case of RNA, the curve followed a pattern similar to that for carbohydrates, a rapid rise followed by a slow decrease. The DNA content increased during the transient state, but at the new steady state the value was significantly lower than before the change in pH.

Figure 49 shows the results for a case in which the reactor pH was changed from 6.40 to 3.00. The biological solids dropped from 450 mg/l to nearly 20 mg/l, and then built up to approximately 600 mg/l. Nearly complete leakage of COD was attained in approximately four detention periods after the shock. COD removal efficiency was recovered rapidly by the acclimated species; the filtrate COD reached a final steady state value of 30 mg/l in approximately fifteen times from the point of shock. As in the previous experiments, the anthrone COD values followed very closely the total COD values, indicating once again that the effluent COD was due chiefly to carbohydrates, and that almost no intermediates and/or end products were produced. Figure 50 shows changes in sludge composition during the experiment. As for the previous acid shock loads, the protein content dropped tremendously; this time from approximately 65 per cent to nearly 20 per cent. During the recovery period there was a slow rise to 26 per cent. The carbohydrate content rose from 15 per cent to approximately 28 per cent, and then slowly receded to nearly 21 per cent. The RNA content also changed; it first increased from 13 per

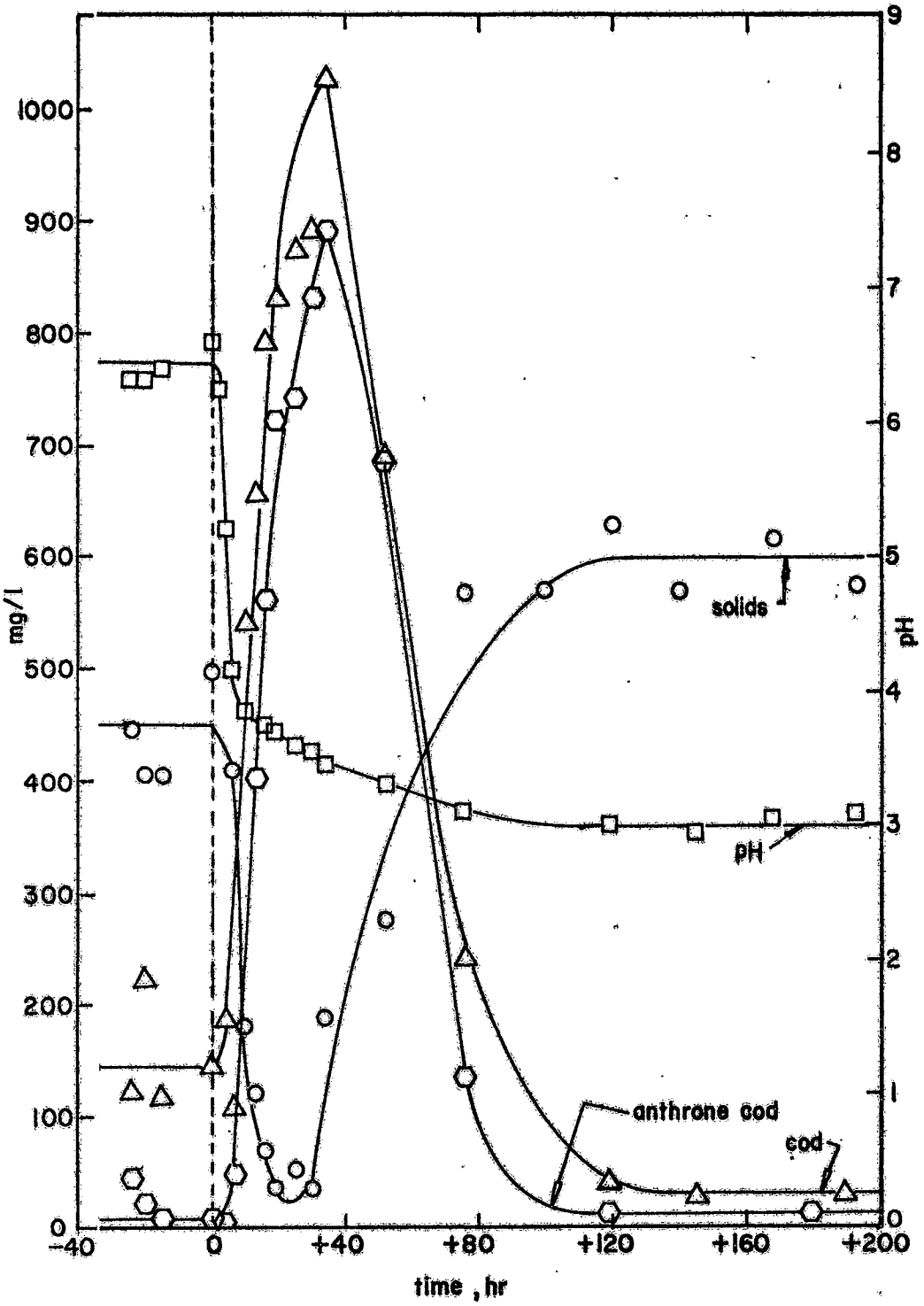

Figure 49 - Response of a system shock loaded from pH 6.40 to 3.00.

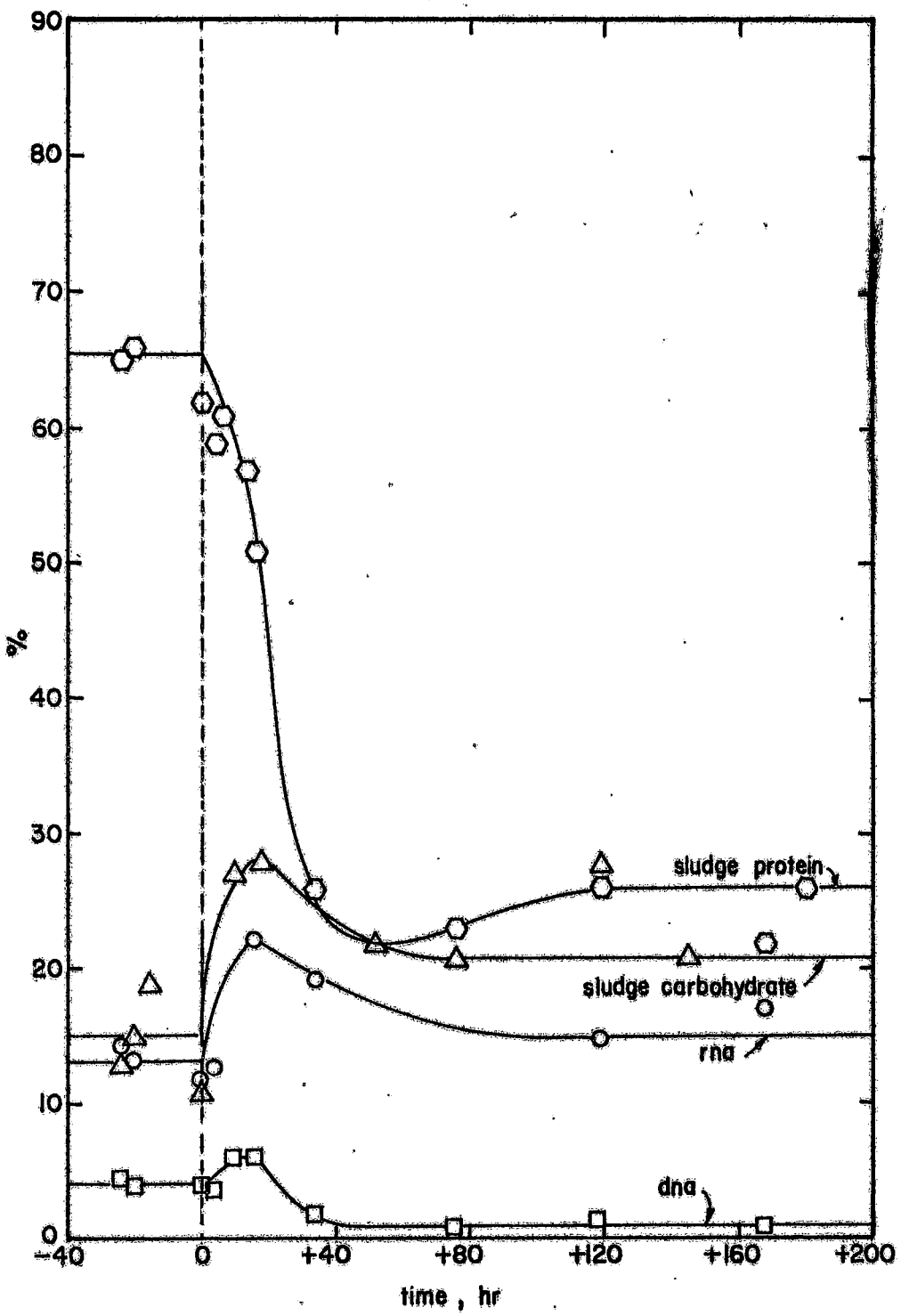

Figure 50 - Changes in sludge composition of a system shock loaded from 6.40 to 3.00.

cent to approximately 22 per cent, and then dropped to nearly 15 per cent. The DNA content also decreased after an initial rise during the cell dilute-out period. The sludge from this unit was analyzed for its elemental composition; the data are given in Figure 51. It is seen that there was no appreciable change in the percentage of carbon and hydrogen; the decrease in percent nitrogen reflects the drop in protein content.

Figure 52 shows the most severe shock load applied, i.e., from pH 6.65 to 2.70. In spite of the comparatively higher level of biological solids in the reactor prior to the shock (approximately 610 mg/l), the severe acid conditions caused essentially all solids to wash out and no recovery was possible.

Table VIII shows the summary data for pre-shock steady state, transient state, and post-shock steady state performance for all pH shock loading experiments. For the three alkaline shock loads in the pH range 7.95 to neutrality, there was no severe transient state response, and the shock loads were easily accommodated by the systems. In the case of acid shock loads, except for experiments in which cells were recycled, the minimum solids at transient state decreased with the severity of the shock load. Time to reach the minimum transient state solids level did not vary a great deal except at the very mild shock load (pH change 6.65 to 6.15) in which case there was evidently a delayed response. Biological solids concentration at the

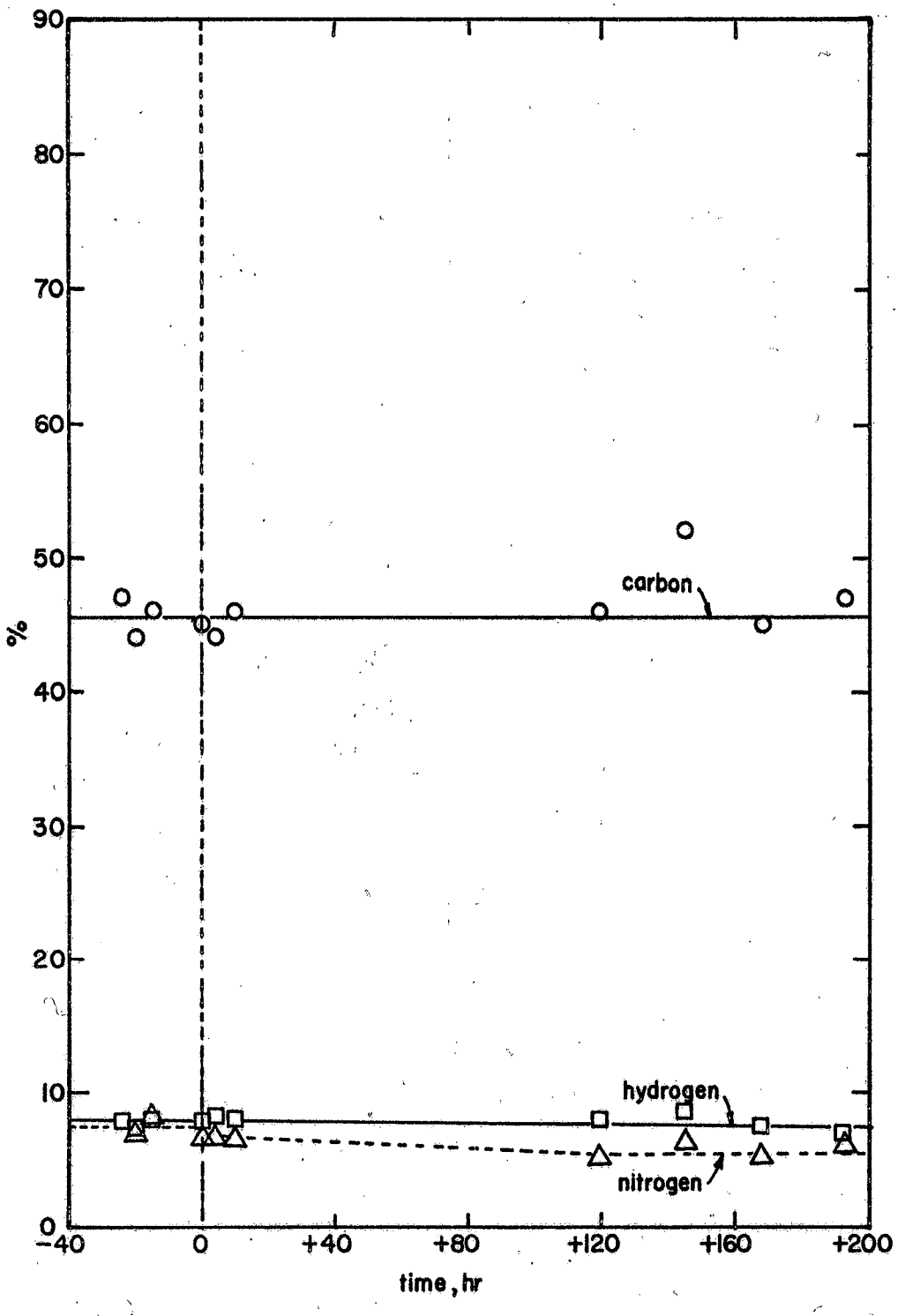

Figure 51 - Elemental composition of sludge from a system shock loaded from pH 6.40 to 3.00.

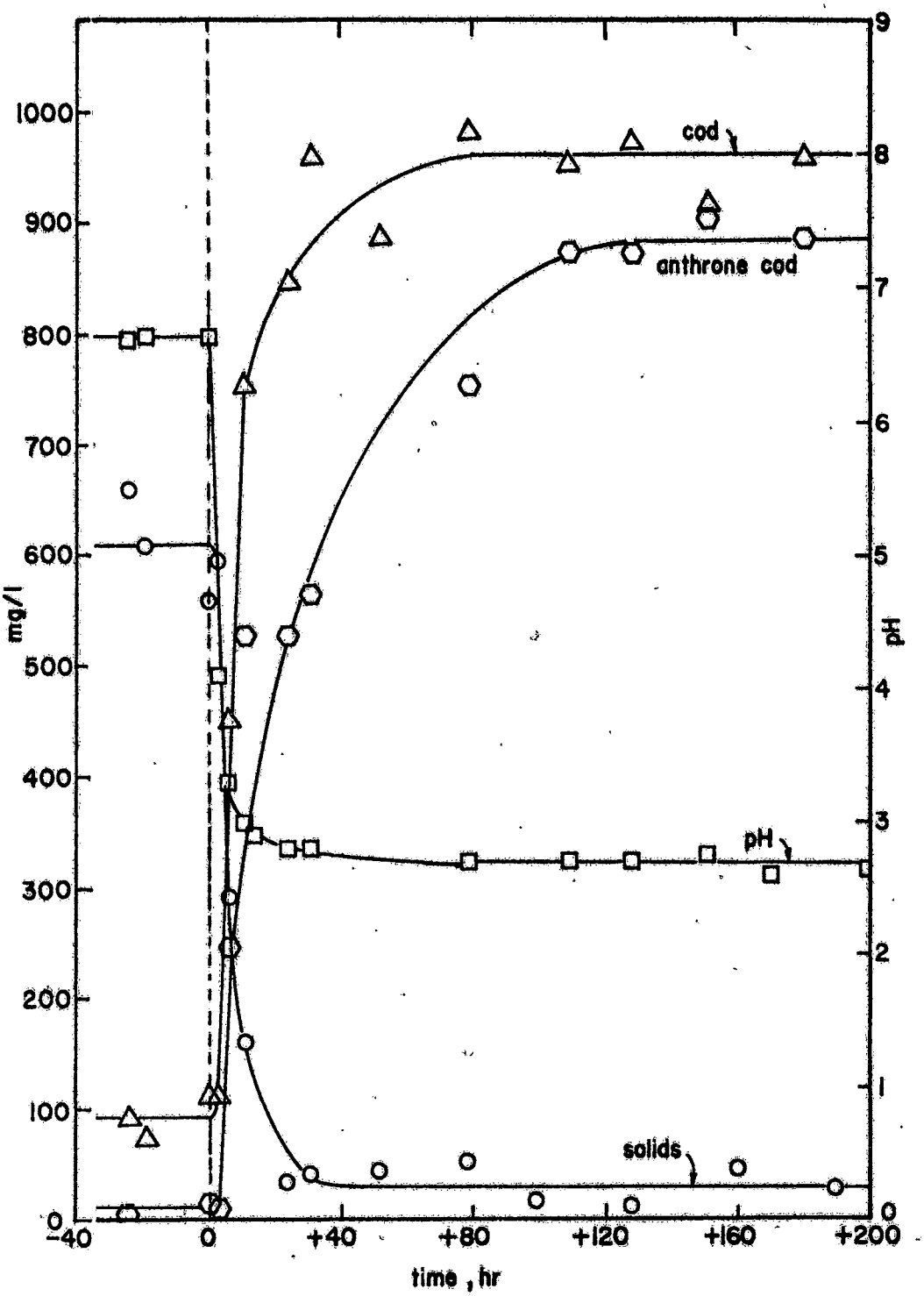

Figure 52 - Response of a system shock loaded from pH 6.65 to 2.70.

TABLE VIII

DATA OF STEADY STATE AND TRANSIENT STATE RESPONSE IN ALL pH SHOCK LOADING EXPERIMENTS

		Alkaline Shocks			Acid Shocks						
		1	2	3	4	5	6	7	8	9	10
Reactor pH before Shock		6.55	6.60*	6.65	6.65	6.70	6.40	6.65*	6.45*	6.60	6.40
Reactor pH at Final Steady State		7.95	7.65	7.25	6.15	5.75	3.50	3.20	3.25	3.15	3.00
Time to reach Final pH	hr.	50	50	30	120	80	150	60	120	96	100
Solids at Initial Steady State	mg/l	464	1150	492	480	530	375	2240	625	432	450
Minimum Solids at Transient State	mg/l	412	-	-	284	350	65	-	140	6	18
Time to reach Minimum Solids	hr.	30	-	-	98	40	34	-	55	32	24
Solids at Final Steady State	mg/l	480	1475	492	680	615	600	908	760	730	600
Time to reach Final Steady State Solids Level	hr.	180	160	0	200	200	170	190	250	150	120
COD at Initial Steady State	mg/l	100	62	52	100	100	225	50	70	66	144
Maximum (Minimum) COD at Transient State	mg/l	60	-	40	234	-	720	150	600	906	1028
Time to reach the Above Value	hr.	130	-	30	98	-	30	23	55	36	34
COD at Final Steady State	mg/l	-	62	74	33	25	32	62	55	120	33
Time to reach Final Steady State	hr.	-	0	140	140	140	150	45	125	120	135

*Systems with Cell Recycle

new steady state varied between 600 and 730 mg/l, and was in all cases higher than the respective pre-shock steady state values. In flow-through systems, the maximum COD at the transient state generally increased with the severity of the shock load, i.e., the greater the change of reactor pH, the greater the COD leakage during the transient state. Neither the time to reach the new steady state COD nor the COD value attained seemed to be in direct relationship to the magnitude of the pH shock. Comparison of columns 7, 8, and 9, which represent nearly the same shock loading situation at different levels of reactor solids, indicates that the transient state effluent COD level was definitely affected by the reactor solids level. The higher the reactor solids level, the lower was the transient state COD.

Table IX shows the steady state parameters preceding and following each shock load. The data for the old steady state conditions shows the variation in the different parameters for essentially identical conditions of operation (except in experiments with cell recycle). These data show that some variations in the different parameters existed during steady state conditions even before shock loading. Because of these natural variations, the effect of shock loading is best analyzed by comparing the response after each change in pH with the corresponding pre-shock steady state. In general, there was an increase in the level of biological solids and the carbohydrate content of the sludge after the shock load. There was a decrease in

TABLE IX

STEADY STATE PARAMETERS PRECEDING AND FOLLOWING pH SHOCK LOADING

Initial Steady State							Final Steady State						
Reactor pH	Solids mg/l	COD mg/l	Anthrone COD mg/l	Protein %	Carbo-hydrate %	DNA %	Reactor pH	Solids mg/l	COD mg/l	Anthrone COD mg/l	Protein %	Carbo-hydrate %	DNA %
Alkaline Shock Loads													
6.55	464	100	4	30	7.5	8.0	7.95	480	–	24	23	15	6.0
6.60*	1150	62	6	49	16	3.5	7.65	1475	62	6	55	17	2.5
6.65	492	52	20	35	7.5	8.0	7.25	492	74	20	29	9	6.0
Acid Shock Loads													
6.65	480	100	36	–	–	4.2	6.15	680	33	10	–	–	3.0
6.70	530	100	25	–	–	–	5.75	615	25	20	–	–	–
6.40	375	225	–	56	15	3.6	3.50	600	32	–	29	25	1.0
6.65*	2240	50	10	52	15	3.5	3.20	910	65	10	39	22	1.5
6.45*	625	70	20	53	13	–	3.25	765	55	5	15	27	–
6.60	432	66	16	51	21	2.5	3.15	730	120	48	28	23	0.5
6.40	450	140	8	65	15	4.0	3.00	600	33	12	26	21	1.0
6.65	609	93	12	–	–	–	2.70	30	960	886	–	–	–

*With Cell Recycle.

effluent COD, effluent anthrone COD, percent sludge protein, and percent sludge DNA as a result of the shock loads.

Table X shows the yield of cells, protein, and carbohydrate based on steady state data prior to and after each shock load. There is a slight increase in the yield of carbohydrates due to the alkaline shock loads; at pH 7.95 the yield actually doubled. In the case of acid shock loads, the increases in yield of cells and carbohydrates were significant, and the change in yield of protein was variable. However, the general tendency appears to be a decrease in the yield of protein.

Table XI shows the yield of cells and COD removal efficiency at the old and new steady states for all experiments. The alkaline shock loads cause very little change in the yield of cells or COD removal efficiency. In the case of acid shock loads, there was a general increase in yield of cells and COD removal efficiency at the new steady state when compared to the old.

During these experiments microscopic observations were made in order to assess any gross changes in microbial predominance at various pH values in the flow-through systems.

Figure 53 shows the general appearance of the floc in a unit operating at a reactor pH of approximately 6.60. It is seen that the predominant forms were rod-shaped bacteria. Bacterial floc particles did exist, but many cells existed in a non-flocculated condition. Some protozoa were also noted at this pH, although none is shown in the picture.

TABLE X

YIELD OF CELLS, PROTEIN AND CARBOHYDRATES AT STEADY STATES BEFORE AND AFTER pH SHOCK LOADING

Initial Steady State				Final Steady State			
Reactor pH	Yield of Cells %	Yield of Cell Protein %	Yield of Cell Carbohydrates %	Reactor pH	Yield of Cells %	Yield of Cell Protein %	Yield of Cell Carbohydrates %
			Alkaline Shocks				
6.55	48	14	3.6	7.95	48	11	7.2
6.65	49	17	3.7	7.25	50	15	4.5
			Acid Shocks				
6.65	50	-	-	6.15	66	-	-
6.70	55	-	-	5.75	59	-	-
6.40	45	25	6.7	3.50	58	17	14
6.60	43	22	9.0	3.15	78	22	18
6.40	49	32	7.3	3.00	58	15	12
6.65*	63	-	-	2.70	30	-	-

*System did not recover after the shock.

TABLE XI

STEADY STATE YIELD OF CELLS AND COD REMOVAL EFFICIENCY BEFORE AND AFTER pH SHOCK LOADING

Initial Steady State			Final Steady State		
Reactor pH	COD Removal Efficiency %	Yield of Cells %	Reactor pH	COD Removal Efficiency %	Yield of Cells %
		Alkaline Shock Loads			
6.55	91	48	7.95	93	48
6.60*	94	-	7.65	94	-
6.65	97	49	7.25	93	50
		Acid Shock Loads			
6.65	91	50	6.15	97	66
6.70	91	55	5.75	98	59
6.40	79	45	3.50	97	58
6.65*	95	-	3.20	94	-
6.45*	93	-	3.25	95	-
6.60	93	43	3.15	89	78
6.40	86	49	3.00	97	58
6.65**	91	63	2.70	9	30

* With Cell Recycle

** System did not recover completely.

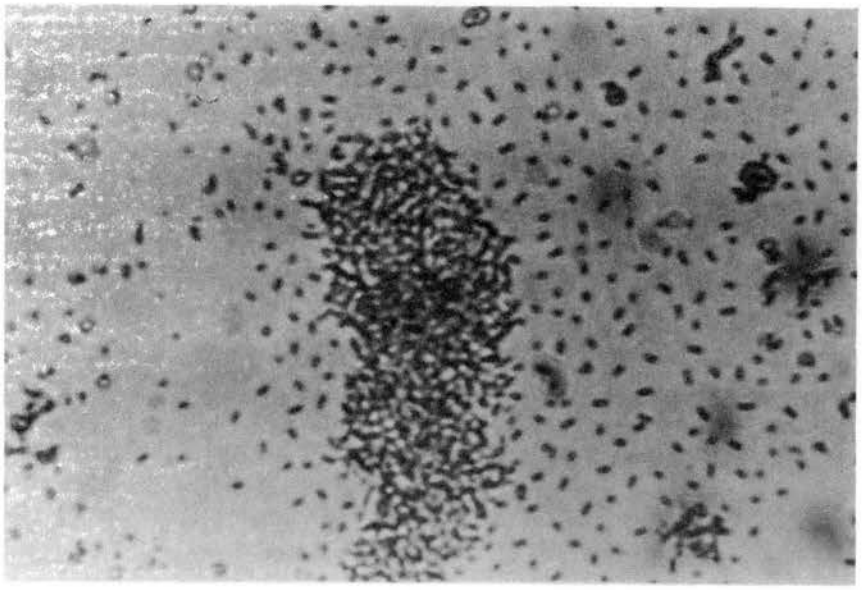

Figure 53 - Photomicrograph of sludge taken from a unit under steady state operation (pH 6.60), magnification, 97x10x3.

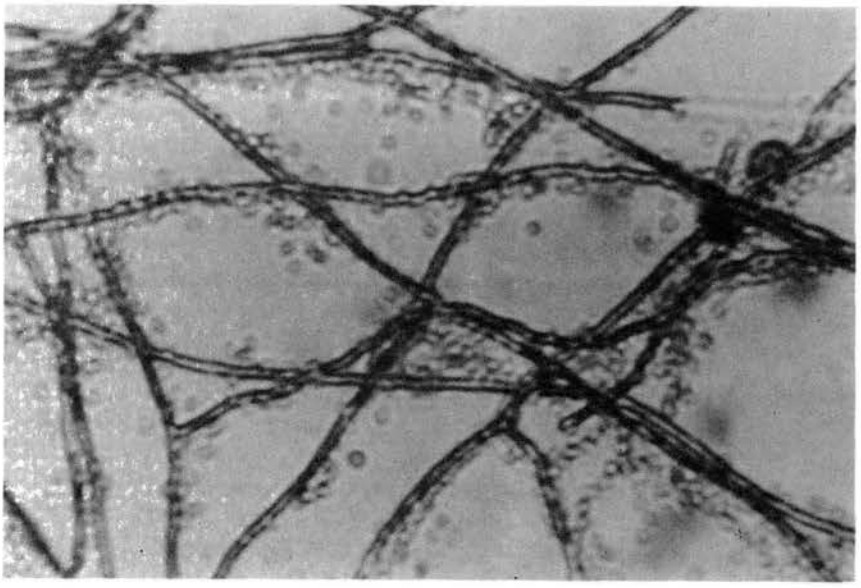

Figure 54 - Photomicrograph of sludge at pH 5.50 (experimental data in Figure 40); magnification, 97x10x3.

Figure 54 shows sludge microorganisms at a reactor pH of approximately 5.50. There was a shift in predominance from individual bacterial cells to filamentous forms which resemble fungi rather than Sphaerotilus. Fewer protozoa were observed at this pH than at pH 6.60.

Figure 55 is a photomicrograph of sludge taken from a reactor at a pH value of approximately 5.00. The general appearance is not much changed from that of Figure 54, which corresponded to a pH of 5.50. However, as seen in Figure 56, when pH was maintained at approximately 3.60, few bacteria were present and filamentous organisms were seen to predominate. Also, at this pH, there was a distinct change in color and texture of the sludge. Some pictures lack clarity; but unfortunately the slides were destroyed, and hence, better pictures are not available.

C. Temperature Shock Loads

Systems initially operating at either four or eight hour detention time and 25°C were subjected to shock loads consisting of an increase or decrease in temperature. In all experiments the change in temperature is plotted along with the response data, in order to facilitate the analysis of the response in relation to rapidity of the temperature change.

1. Decrease in Temperature

Figure 57 shows the response for a system operating at an eight-hour detention time which was shock loaded from a temperature of 25°C to 8°C. This was the most severe cold

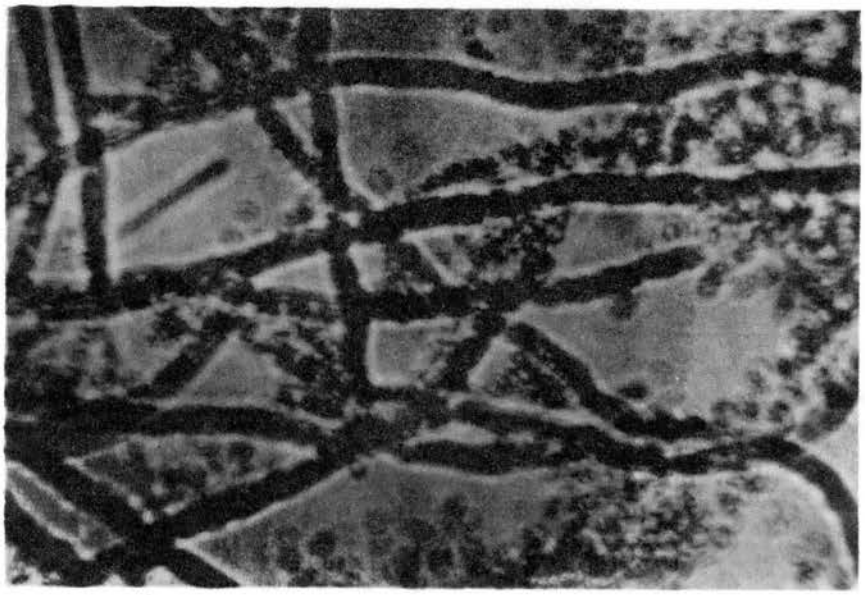

Figure 55 - Photomicrograph of sludge growing at pH 5.00 (experimental data in Figure 40), magnification, 97x10x3.

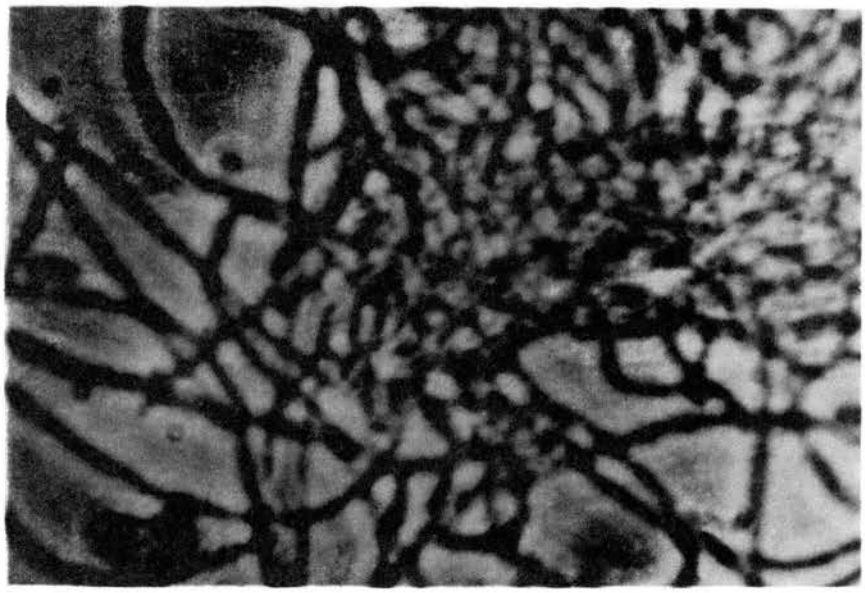

Figure 56 - Photomicrograph of sludge acclimated to pH 3.60 (experimental data in Figure 41), magnification, 97x10x3.

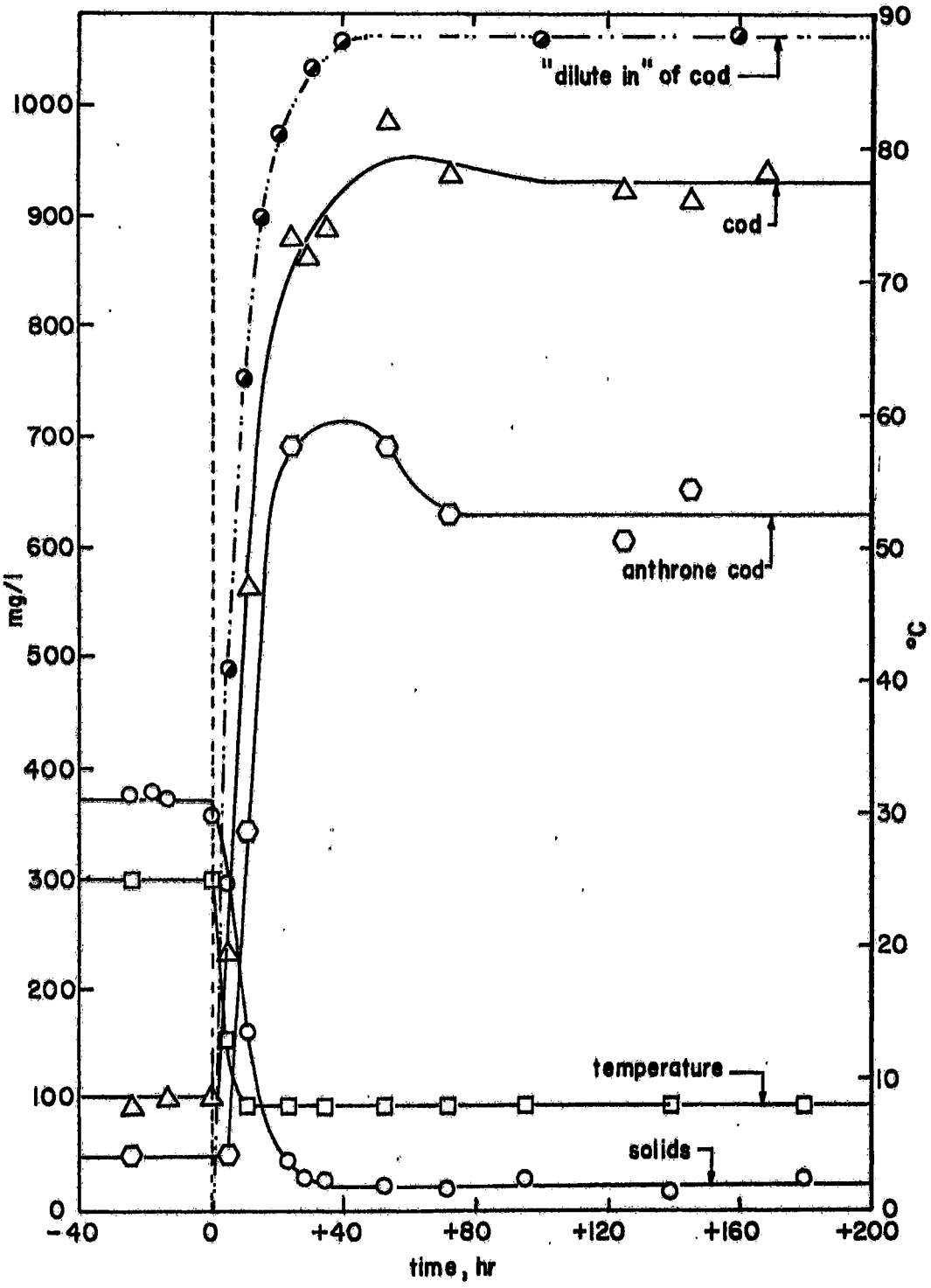

Figure 57 - Response of a system shock loaded from 25°C to 8°C (detention time 8 hours).

shock that was applied, and it is apparent that the system could not respond satisfactorily to this severe shock load. The biological solids decreased from the point of application of the shock. In approximately four detention periods the solids concentration reached a very low value of approximately 24 mg/l, and it never recovered during the twenty-five detention periods after the shock. The filtrate COD increased steadily from nearly 100 mg/l to approximately 930 mg/l. The dilute-in curve indicates the level of COD that would have been present in the reactor if the metabolic activity of the cells completely ceased when the shock was initiated. Comparison of the curves of dilute-in, effluent COD, and anthrone COD indicates that most of the input COD appeared in the effluent, and a certain amount of the COD was due to metabolic intermediates; however, a large proportion of the COD was contributed by carbohydrates in the effluent.

Figure 58 shows results for the same temperature shock load applied to a system operating at a detention time of four hours. The dilute-out of solids was more severe in this case, and there was no indication of recovery. There was a rapid rise in effluent COD concentration, the course of which was very near that of the dilute-in curve. The anthrone COD curve indicates that most of the dissolved organic matter leaving the reactor was in the form of carbohydrates. However, concerning that portion of the COD which was attributable to metabolic intermediates and/or

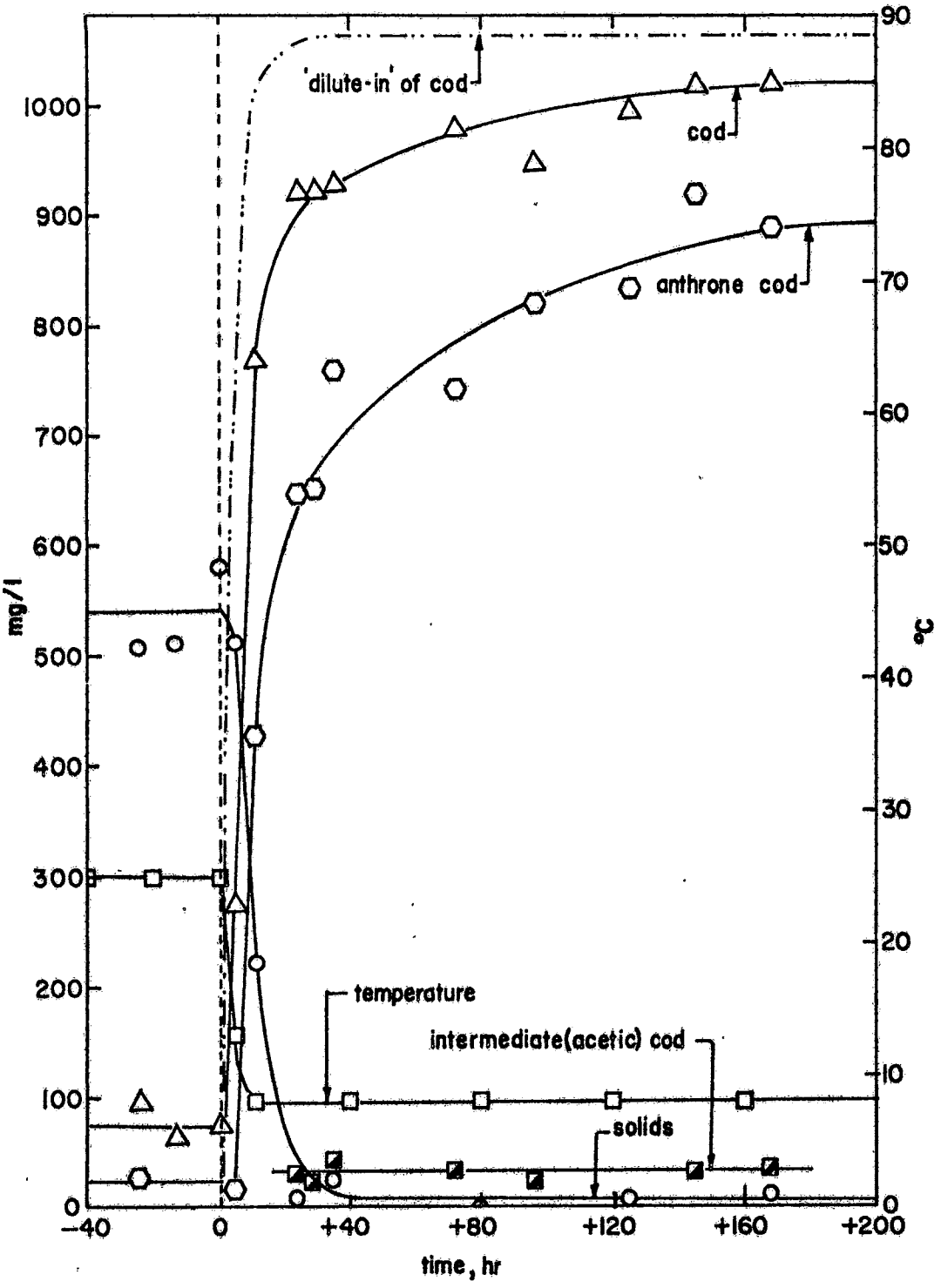

Figure 58 - Response of a system shock loaded from 25°C to 8°C (detention 4 hours).

end products, gas chromatographic analysis indicated that traces of acetic acid (approximately 30 mg/l) were present. The temperature drop from 25 to 8°C was effected in twelve hours (three detention times), i.e., it was by no means immediate; however, the system was not able to respond satisfactorily.

Figure 59 shows the biochemical response of a system at a detention time of eight hours, shock loaded from a temperature of 25 to 17.5°C. The temperature change was effected in six hours, i.e., three-fourths of the detention time. From a steady state value of 540 mg/l, the biological solids level dropped to approximately 420 mg/l in about twenty-two detention times, and then slowly rose to nearly 600 mg/l. During the transient condition there was a significant increase in total COD from approximately 60 mg/l to nearly 180 mg/l in about four detention times. The anthrone COD value did not increase. Figure 60 shows the changes in sludge composition during the experiment. During the transient state the protein content of the sludge increased by eight per cent, and the carbohydrate content decreased by four per cent. However, at the new steady state, the values were very near their pre-shock values. The RNA content rose from ten per cent to approximately fourteen per cent, and then returned to the previous level; the change in DNA was very small.

Figure 61 shows the response to the same temperature change applied to a system operating at a detention time of

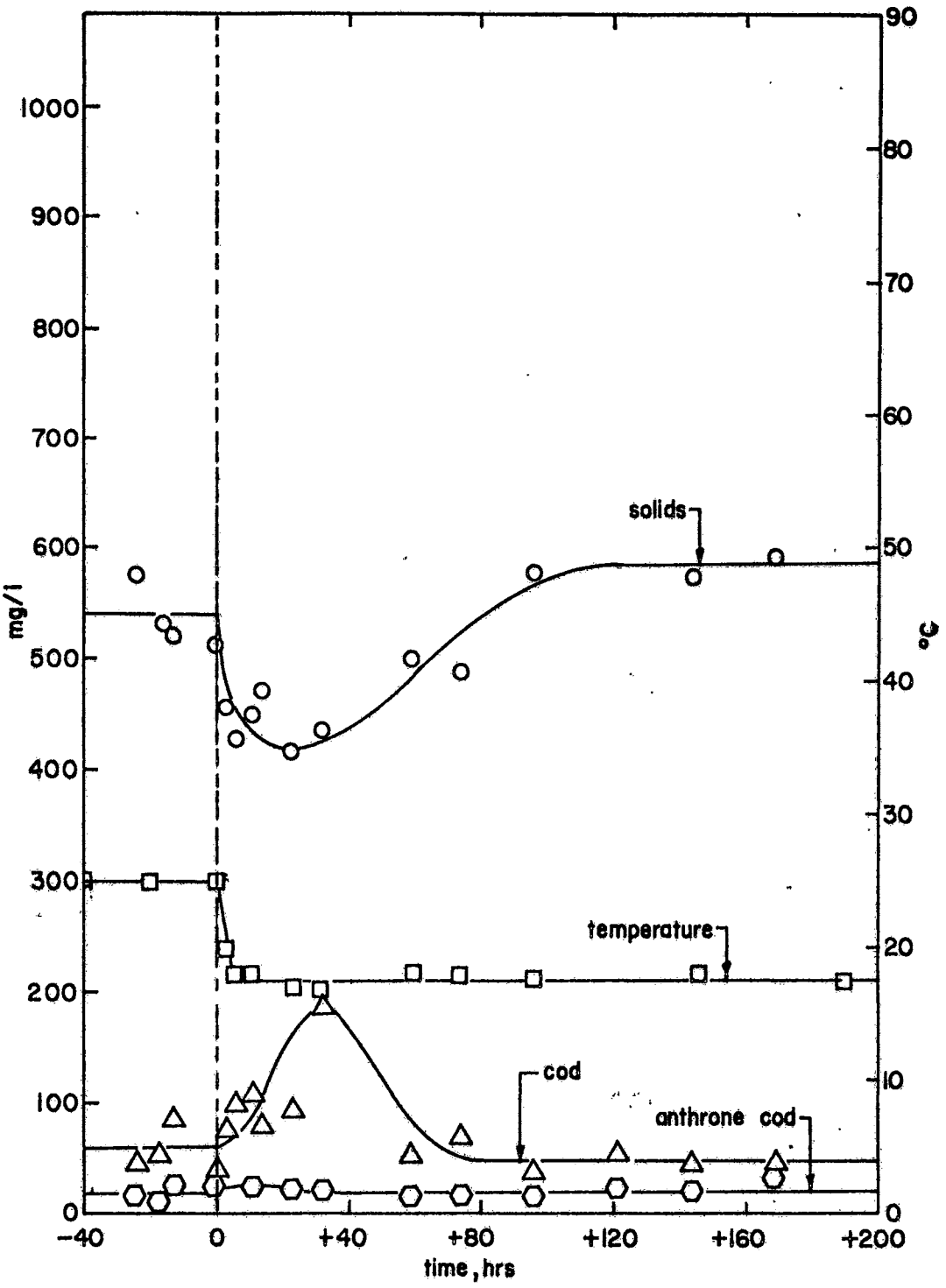

Figure 59 - Response of a system shock loaded from 25°C to 17.5°C (detention 8 hours).

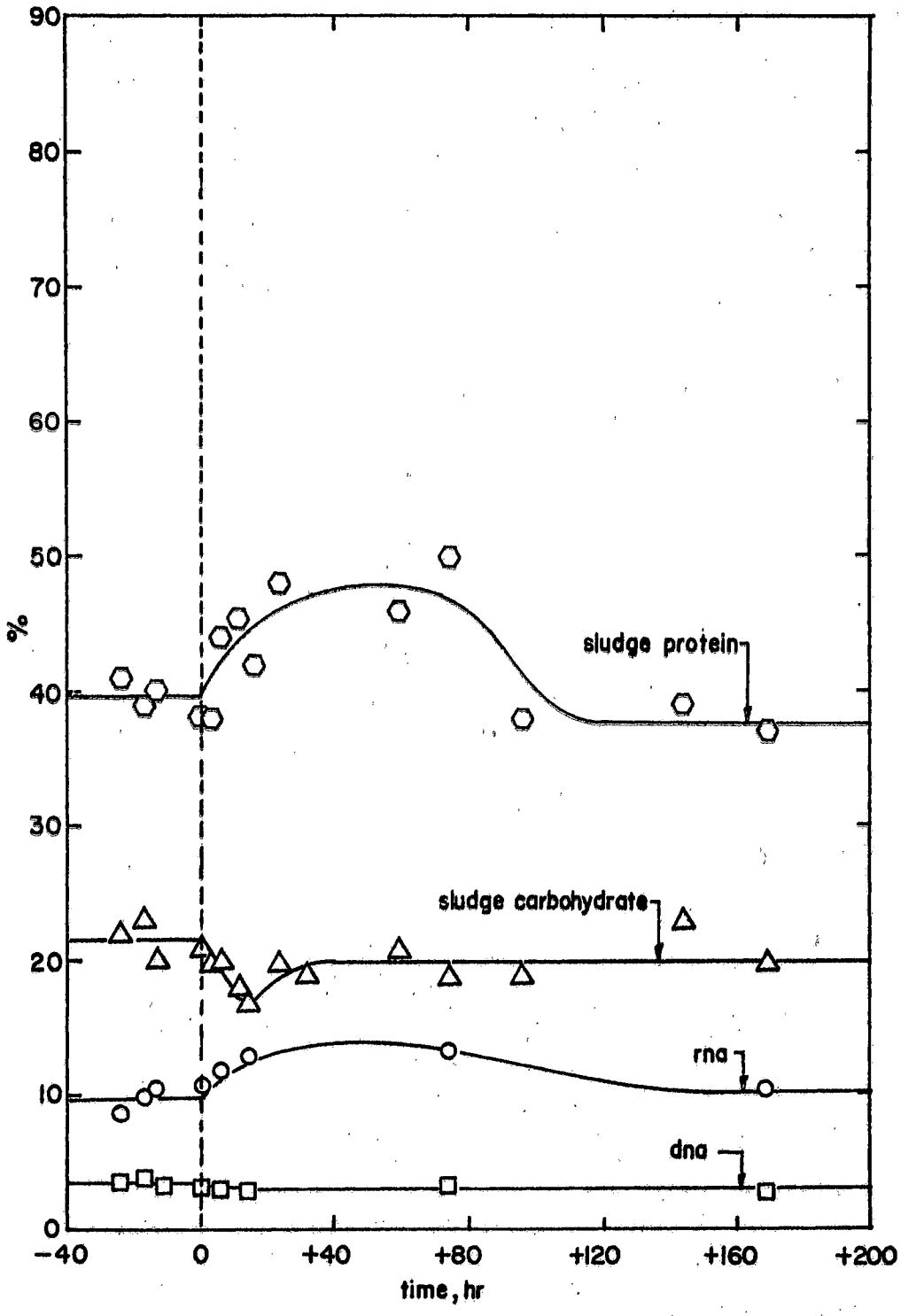

Figure 60 - Changes in sludge composition of a system shock loaded from 25°C to 17.5°C (detention 8 hours).

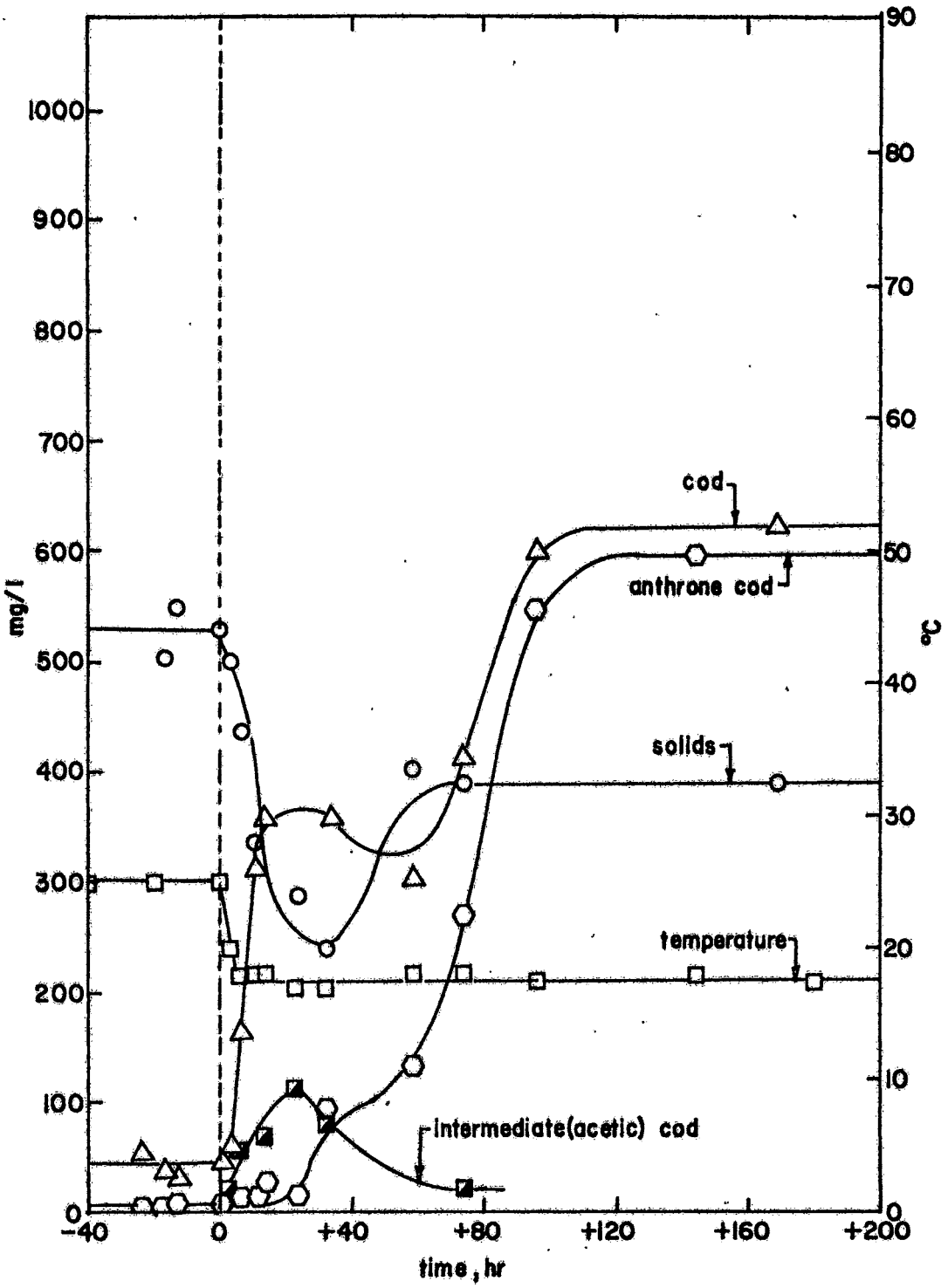

Figure 61 - Response of a system shock loaded from 25°C to 17.5°C (detention 4 hours).

four hours. Comparing these results with those shown in Figure 59 (8-hour detention time), it can be seen that the operating detention time (growth rate) affected the response pattern. The temperature drop from 25°C to 17.5°C was effected in six hours, or 1.5 detention periods. The solids concentration decreased from approximately 510 mg/l to 240 mg/l in eight detention times, and then slowly rose to nearly 390 mg/l within eighteen detention periods (72 hours) from the point of shock. The COD of the effluent appeared to have risen in two stages. During the first stage it rose from approximately 45 mg/l to nearly 360 mg/l, and after some time it rose again to approximately 625 mg/l. By comparing the anthrone COD curve and the total COD curve, it can be seen that during the first stage of COD increase considerable amounts of metabolic intermediates and/or end products were released into the system. However, during the second stage, a major portion of the COD registered as carbohydrates. Analysis of samples by gas-liquid chromatography indicated that part of the intermediates released during the first stage was acetic acid. The acetic acid concentration reached a maximum of 114 mg/l expressed as COD. Figure 62 shows the changes in sludge composition during this experiment. The protein percentage values fluctuated, but in general there appeared to be a slight increase initially and afterward some decrease. The reverse effect was apparent for the carbohydrate content of the sludge. The carbohydrate content decreased from approx-

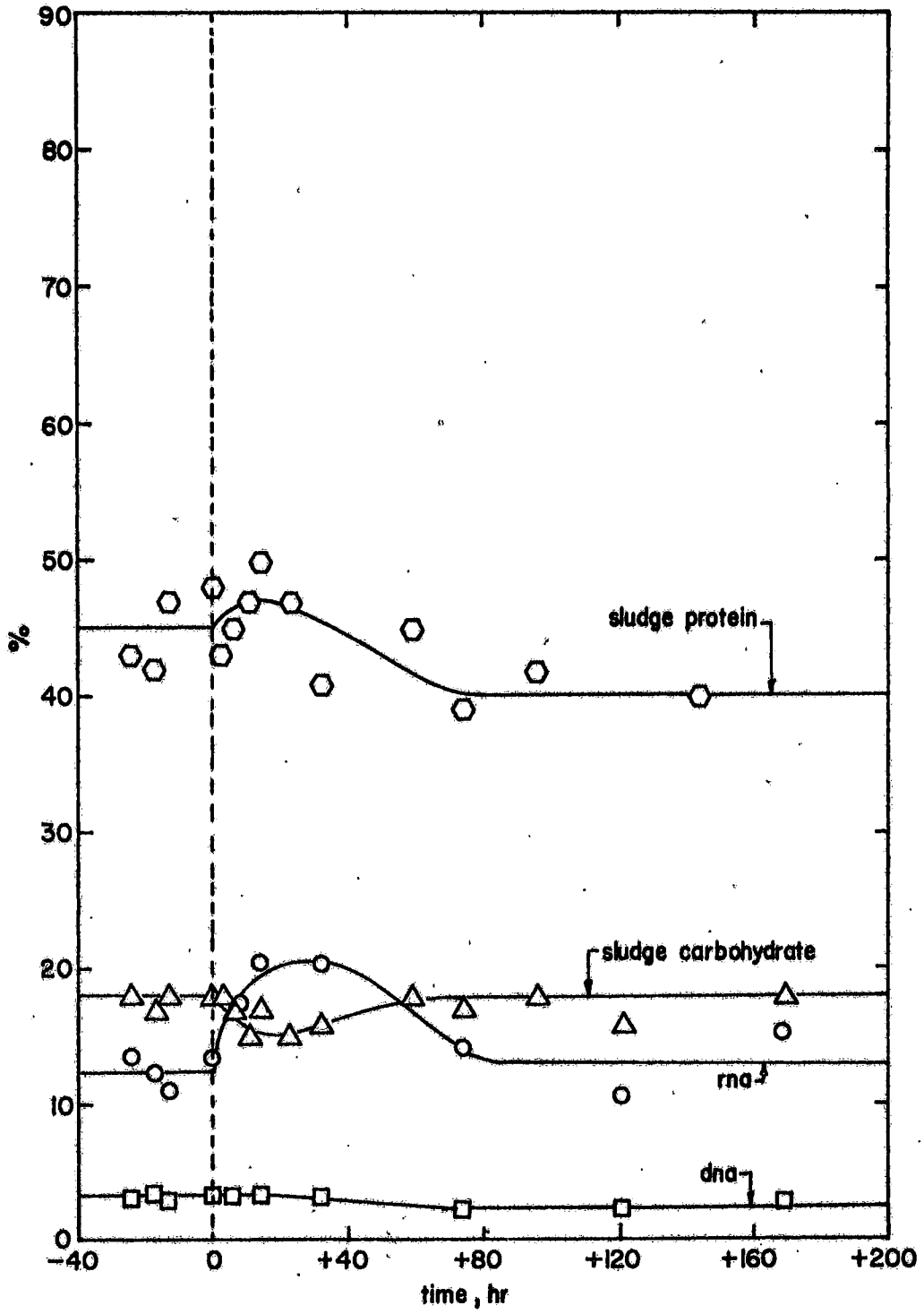

Figure 62 - Changes in sludge composition of a system shock loaded from 25°C to 17.5°C (detention 4 hours).

imately 18 per cent to nearly 15 per cent during the transient state, and then gradually returned to 18 per cent. The RNA content increased from approximately 12 per cent to 21 per cent during the unsteady conditions, and then slowly returned to an average value of approximately 13 per cent. There was a very small decrease in the DNA content. It is noted that the changes in cell composition shown in Figure 62 are very similar to those shown in Figure 60 for a system subjected to the same temperature shock, but at a detention time of eight hours.

2. Increase in Temperature

Figure 63 depicts the system response to an increase in temperature from 25 to 36°C. The reactor detention time was eight hours, and the 11 degree increase in temperature was effected in a period of five detention times. This was rather a gradual change, and the system responded successfully. There was a very slow lowering of the reactor solids level from approximately 490 mg/l to nearly 420 mg/l in a period of fourteen detention times, followed by an increase to approximately the previous level. The total transient response time with respect to solids was twenty-two detention times, but the decrease in solids concentration was rather small. Also, the filtrate COD was only slightly affected; the values fluctuated between 40 and 80 mg/l. The anthrone COD values were very low. Figure 64 shows the changes in sludge composition caused by this shock load. Complementary changes in protein and carbo-

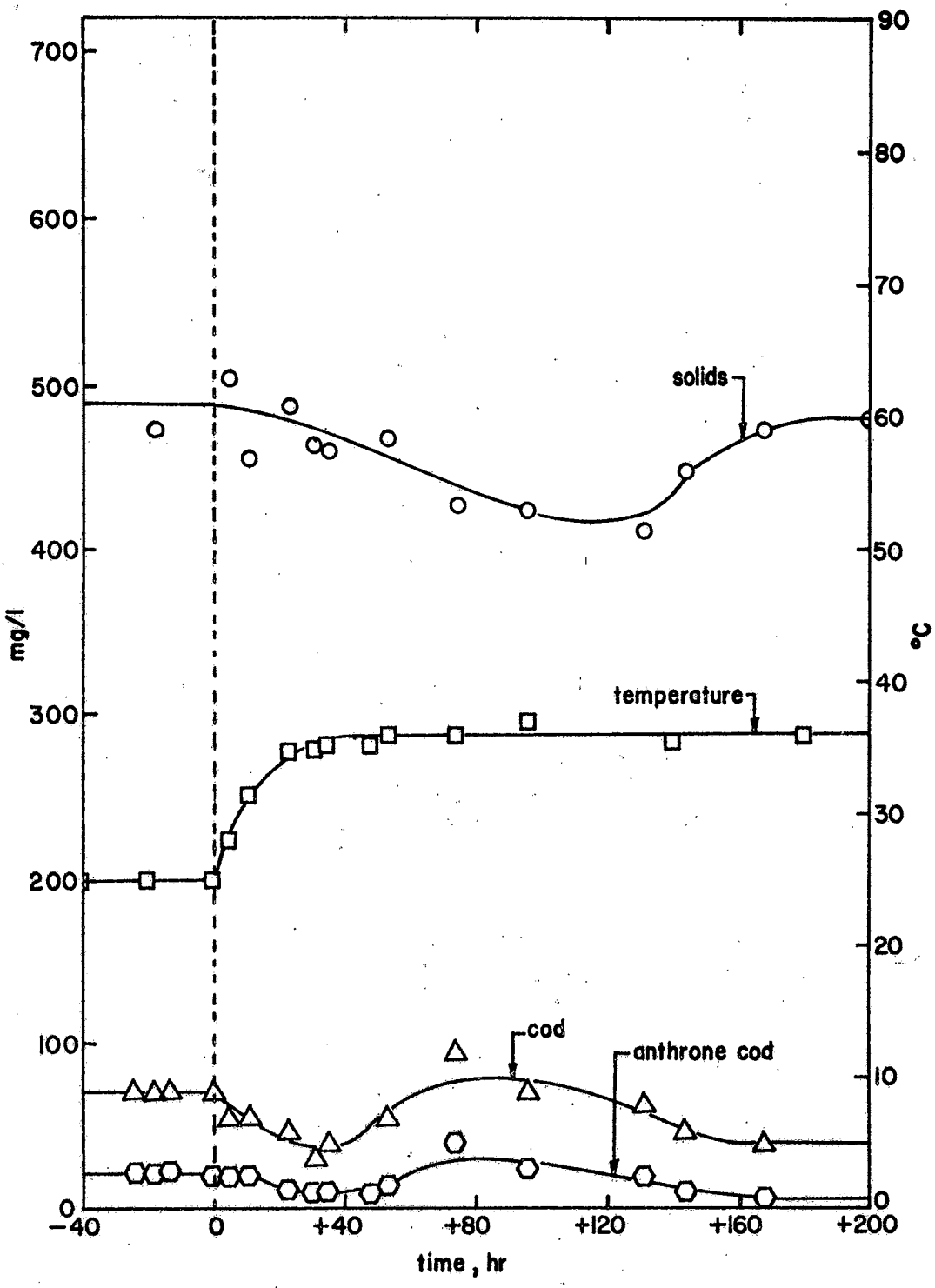

Figure 63 - Response of a system shock loaded from 25°C to 36°C (detention 8 hours).

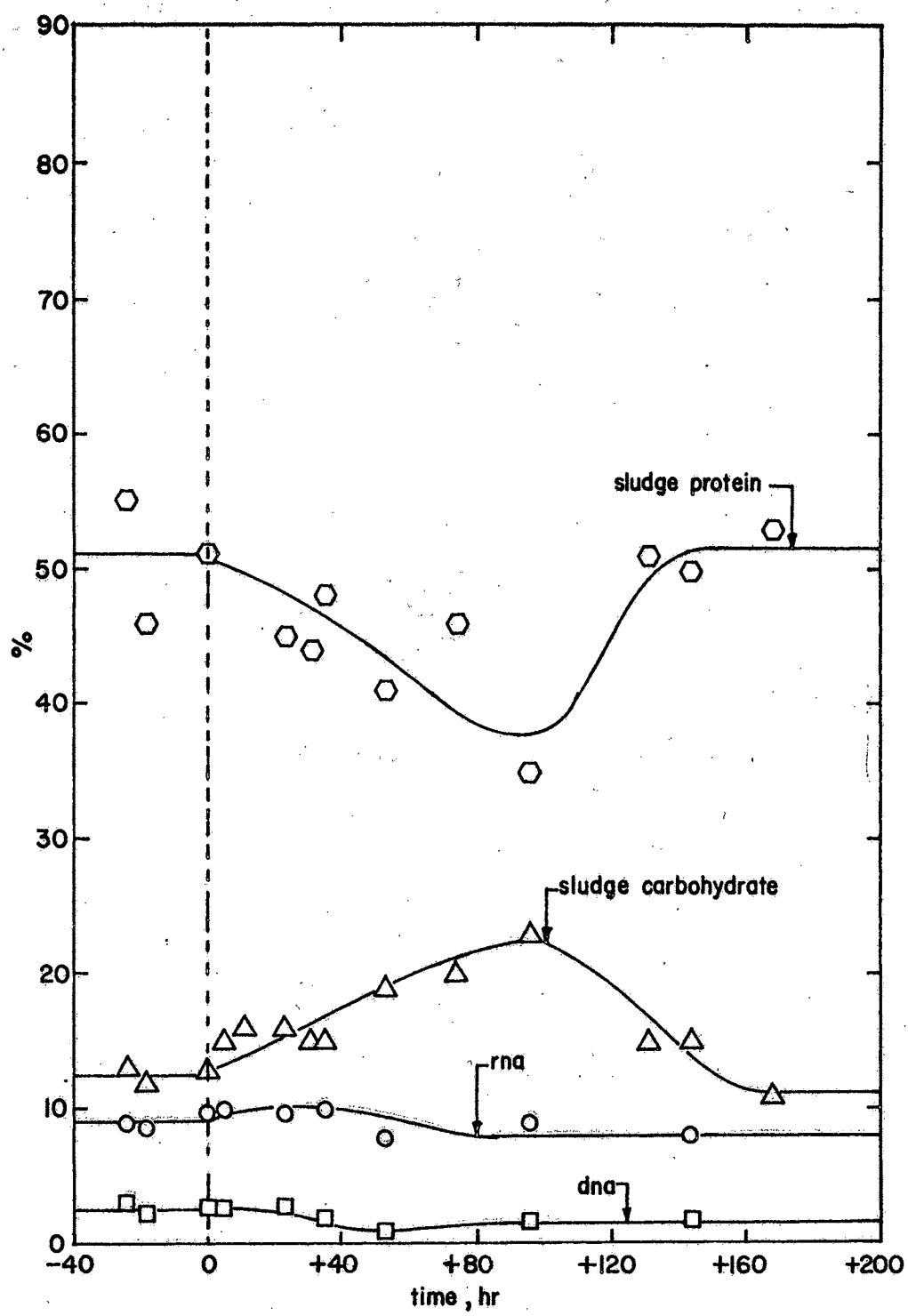

Figure 64 - Changes in sludge composition of a system shock loaded from 25°C to 36°C (detention 8 hours).

hydrate content are quite apparent. The increase in temperature caused an initial lowering of the protein content by approximately 10 per cent, followed by a return to the previous value. The carbohydrate content rose from approximately 12.5 per cent to a maximum of 22 per cent, and then gradually returned to the original value. The changes in RNA and DNA content were small.

Figure 65 shows the same shock loading conditions imposed on a system operating at a detention time of four hours. The temperature change from 25 to 36°C was completed in ten detention times (forty hours). The biological solids concentration underwent wide fluctuations during the temperature transient, and subsequently dropped to a steady state value of approximately 425 mg/l from the pre-shock steady state value of nearly 575 mg/l. However, there was no significant rise in COD of the filtrate. The anthrone COD was very low at all times. Thus, the data of Figures 63 and 65 indicated that an increase from 25 to 36°C was easily accommodated at both four and eight-hour detention times.

Figure 66 shows the changes in sludge composition for the experiment shown in Figure 65. There was a pronounced increase in protein content of the sludge (from 43 to 55 per cent). The initial depression in the percent protein curve observed for the eight-hour detention time (Figure 64) was not evident for the four-hour detention time (Figure 66). The carbohydrate content decreased somewhat, as did the RNA

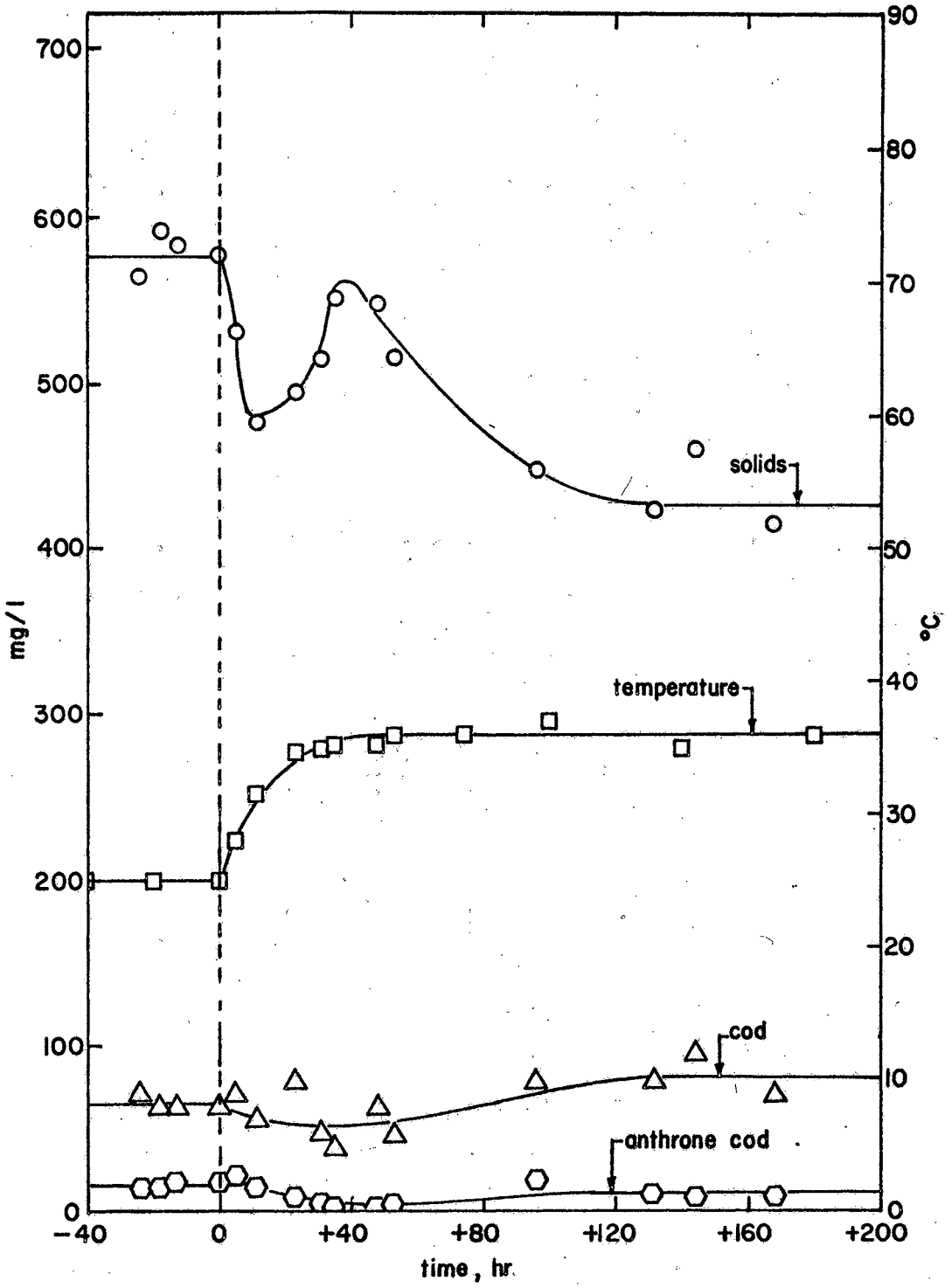

Figure 65 - Response of a system shock loaded from 25°C to 36°C (detention 4 hours).

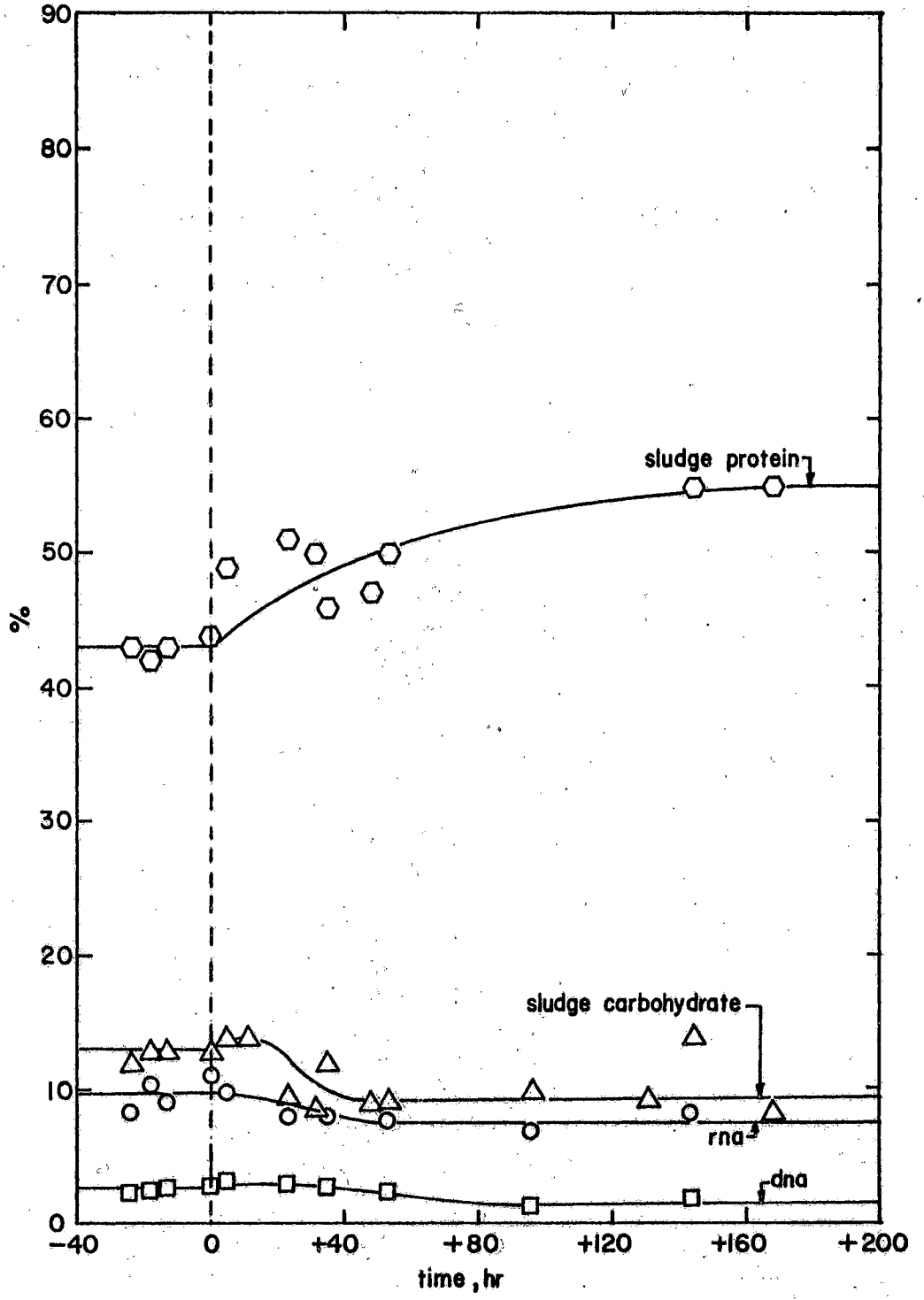

Figure 66 - Changes in sludge composition of a system shock loaded from 25°C to 36°C (detention 4 hours).

and DNA content.

Figure 67 shows the response of a system subjected to a more severe temperature shock. The detention time was eight hours, and the temperature was increased approximately two-fold (25 to 47°C). The temperature change was effected in approximately 3.5 detention times (26 hours). The system experienced a quite deleterious response. The biological solids decreased from approximately 510 mg/l to nearly 90 mg/l, and then rose to a significantly lower new steady state level of approximately 310 mg/l. During the period of depression in the solids level the total effluent COD rose to an abnormally high value of nearly 920 mg/l, but rapidly returned to a lower value in response to the recovery in solids concentration. The recovery of the system required approximately seven detention times. From a comparison of the total and anthrone COD values, it can be seen that the major portion of the effluent COD was not in the form of carbohydrates. Analysis by gas-liquid chromatography did not indicate the presence of any volatile acids, and hence the metabolic intermediates and/or end products that were released were in some form other than carbohydrates or volatile acids. Figure 68 shows changes in sludge composition during the experimental period. The protein content dropped slightly and then gradually rose to approximately the same levels as before shock. The carbohydrate content fluctuated widely during the transient conditions, and then attained a low value of nearly 8 per cent,

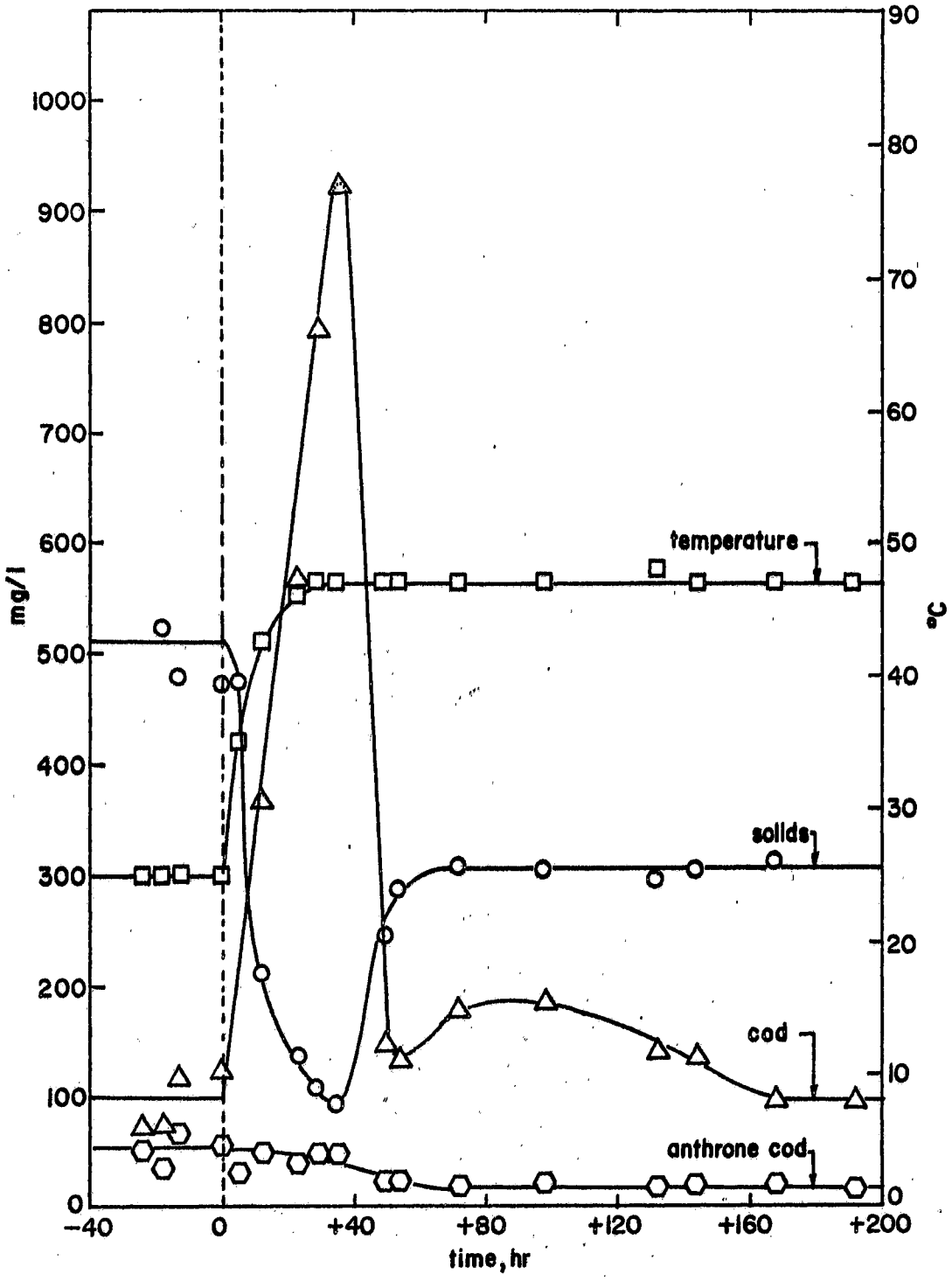

Figure 67 - Response of a system shock loaded from 25°C to 47°C (detention 8 hours).

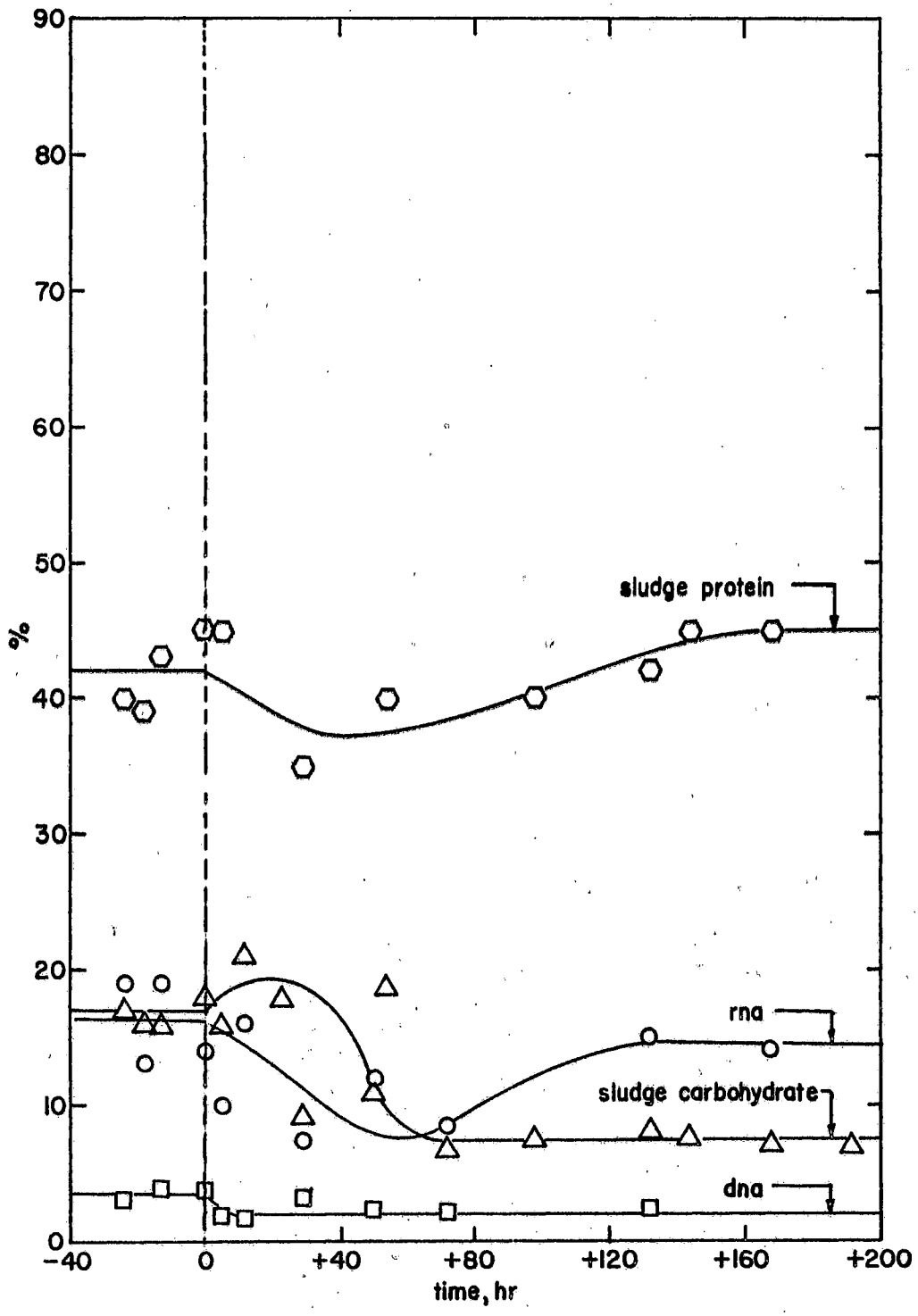

Figure 68 - Changes in sludge composition of a system shock loaded from 25°C to 47°C (detention 8 hours).

which is approximately half of the value prior to the shock. The RNA content decreased during the transition state, and then rose slowly. There was a slight decrease in the DNA content. The most significant change in sludge composition was the large decrease in carbohydrates at the elevated temperature.

Figure 69 shows the reaction of a steady state system, operated at a detention time of four hours, when subjected to a change in temperature from 25 to 47°C. The system experienced a severe loss of substrate removal efficiency during the transient state. The change in temperature was effected in approximately seven detention times. The biological solids decreased from a steady state value of approximately 515 mg/l, and were nearly washed out of the aerator. The solids concentration fell to approximately 12 mg/l. It is surprising that after staying at this low value for nearly forty hours, a rapid acclimation took place and the solids level rose to a new steady state value of approximately 320 mg/l. COD removal efficiency was rapidly recovered as a result of the rise in solids concentration. The anthrone COD returned to its previous value. It is interesting to note that in this case the leakage of COD during the transient was due to leakage of the carbohydrate substrate; however, for the same temperature change the system operated at a detention time of eight hours (see Figure 67) experienced COD leakage due almost entirely to metabolic intermediates and/or end products. Figure 70

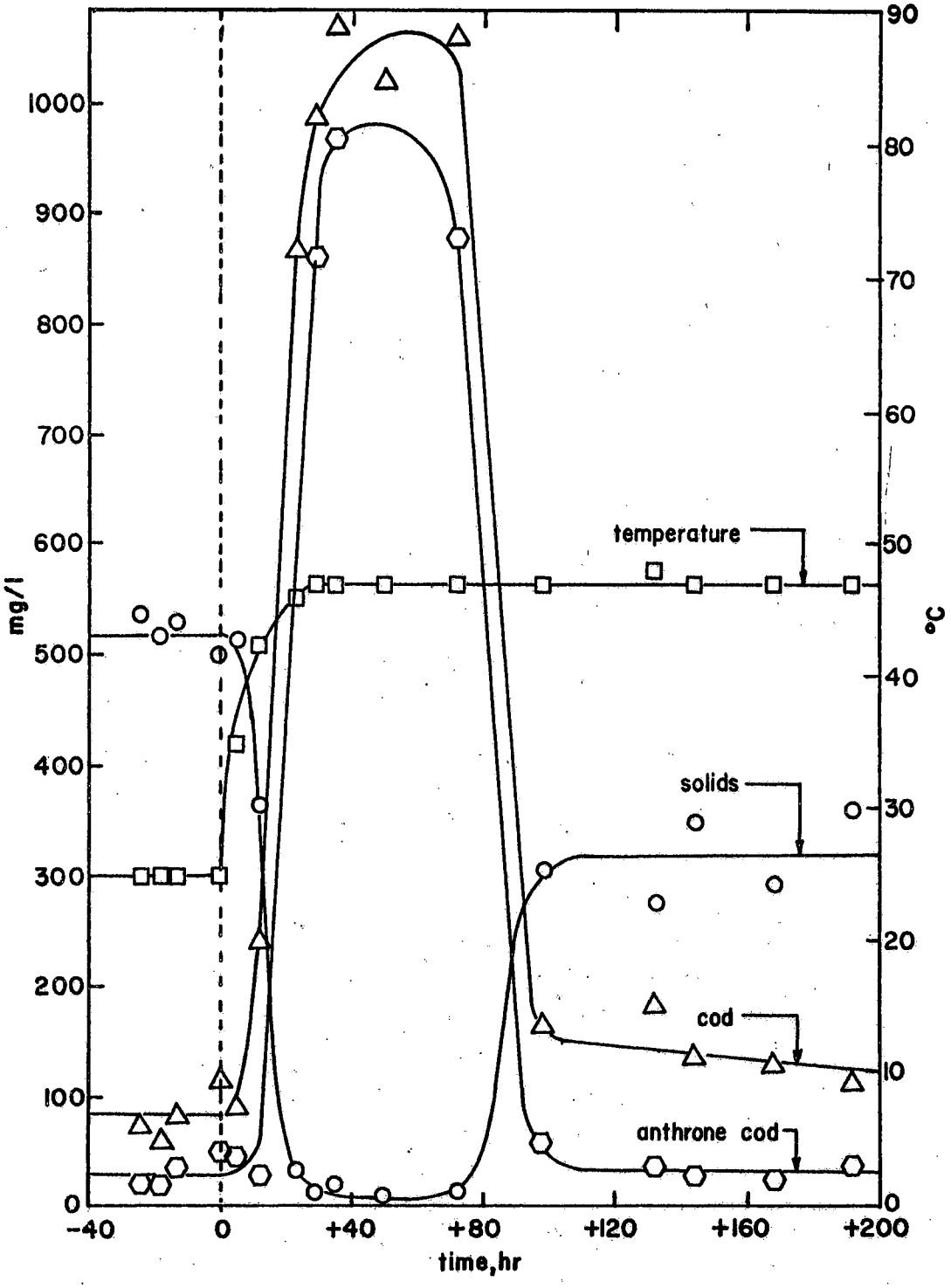

Figure 69 - Response of a system shock loaded from 25°C to 47°C (detention 4 hours).

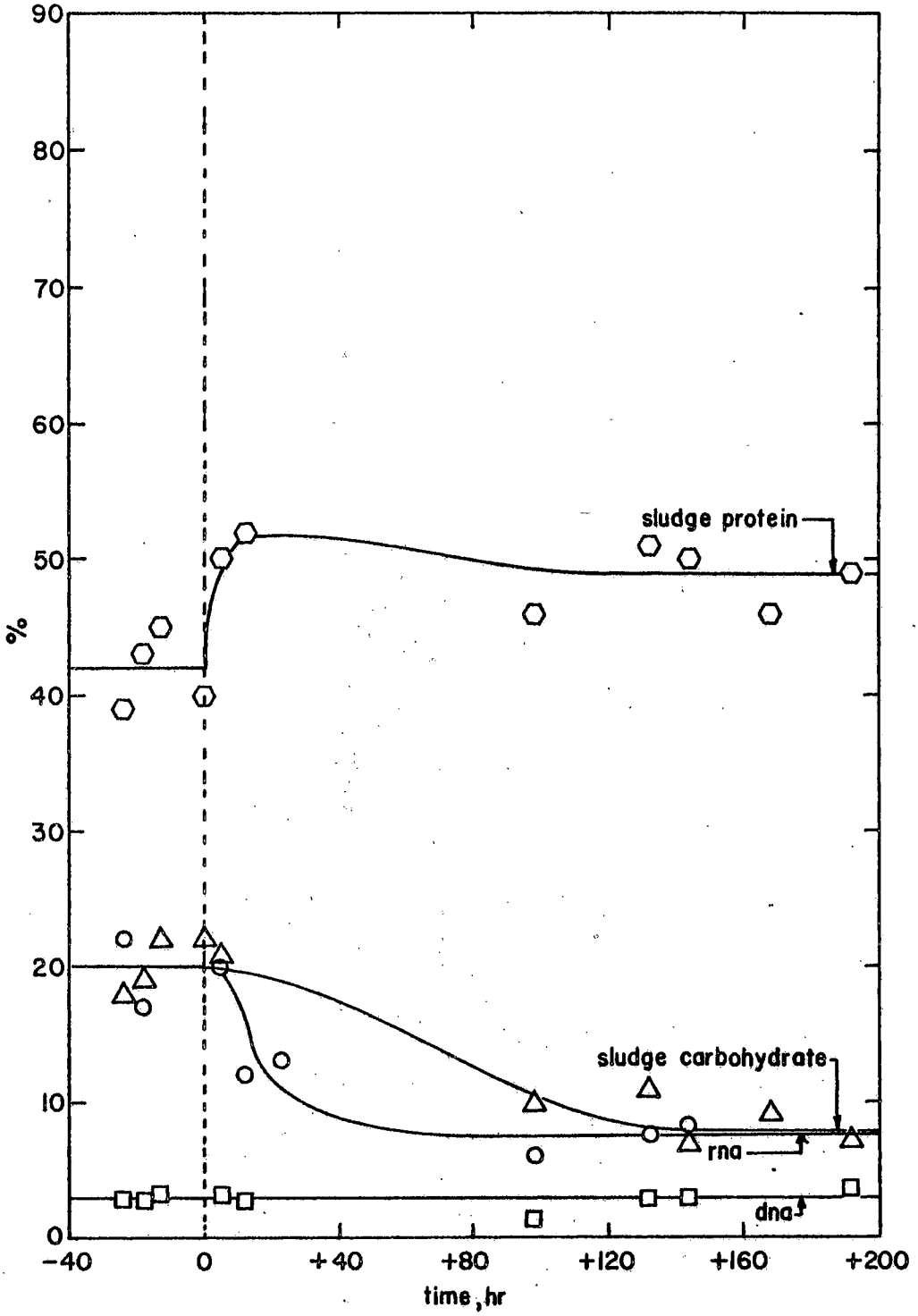

Figure 70 - Changes in sludge composition of a system shock loaded from 25°C to 47°C (detention 4 hours).

shows changes in sludge composition during the experiment. In general, the response is similar to that seen in Figure 66 for a less severe shock load under otherwise identical operating conditions; however, in this case, the decrease in carbohydrate and RNA content is more pronounced, and the increase in protein content is smaller.

Figure 71 shows the most severe temperature shock that was applied during the current investigations. The temperature change (25 to 57.5°C) was effected during a time interval equivalent to six detention periods. The biological solids decreased nearly to the point of complete washout, but at the end of approximately ten detention periods thermophiles grew up and reached a new steady state solids level of approximately 180 mg/l. Upon application of the shock load, the COD curve rose rapidly, reaching a value equivalent to that in the influent COD. As the solids level increased, the COD decreased and attained a new steady state level of 450 mg/l. However, the purification efficiency is not comparable with values generally expected of an activated sludge plant. During the transient state, the anthrone COD curve closely parallelled the COD curve, a result which is in total disagreement with the occurrence shown in Figure 67 for a less severe shock load. However, it is important to note that considerable quantities of metabolic intermediates and/or end products were released in the final steady state. Analysis of volatile fatty acids (gas-liquid chromatography) indicated that acetic

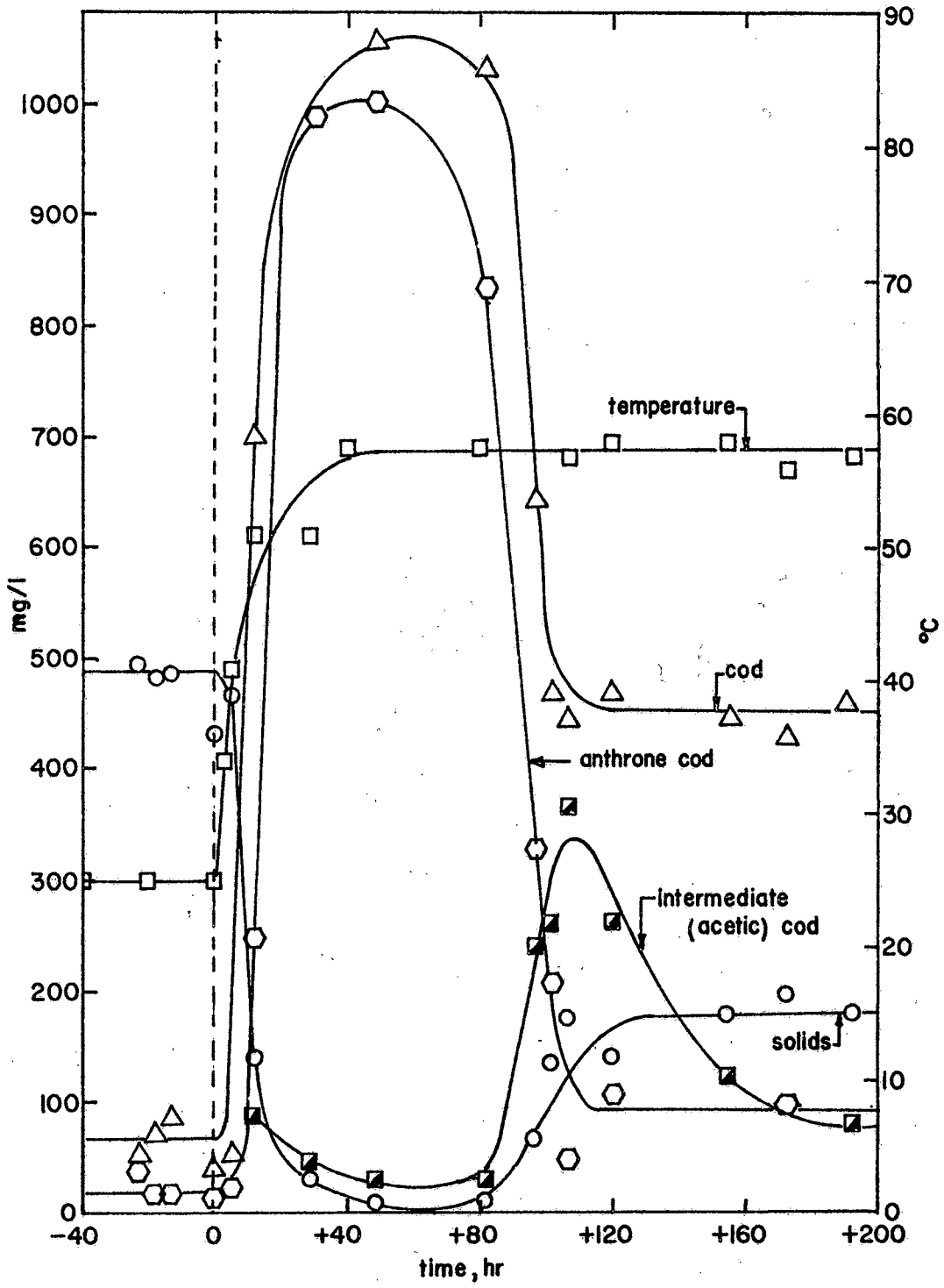

Figure 71 - Response of a system shock loaded from 25°C to 57.5°C (detention 8 hours).

acid was one of the compounds that was being produced, and that it reached maximum concentration at the end of the transition state.

Figure 72 shows the response to a temperature shock from 25 to 57.5°C at a detention time of four hours. The temperature shift was effected during a period of twelve detention times. It is noted that the original steady state solids level was rather low (an average of 310 mg/l), and solids were washed out during three detention periods after initiation of the shock. For approximately twenty detention periods biological solids were, for all practical purposes, absent from the system. However, it is evident that some organism did persist, because the biological solids level eventually rose to approximately 65 mg/l and appeared to attain a steady state. As in the previous experiment, the system could not attain at this high temperature the efficiency it exhibited at 25°C. At the new steady state the effluent COD was primarily due to carbohydrates.

It is to be emphasized that dissolved oxygen measurements were made periodically on all systems. In all experiments the level of dissolved oxygen was always higher than 3 mg/l.

Table XII shows the changes in effluent COD and biological solids concentration in response to the changes in temperature for all experiments. At the shock temperature of 8°C the system broke down completely. At the 17.5°C temperature the system at four-hour detention time did not

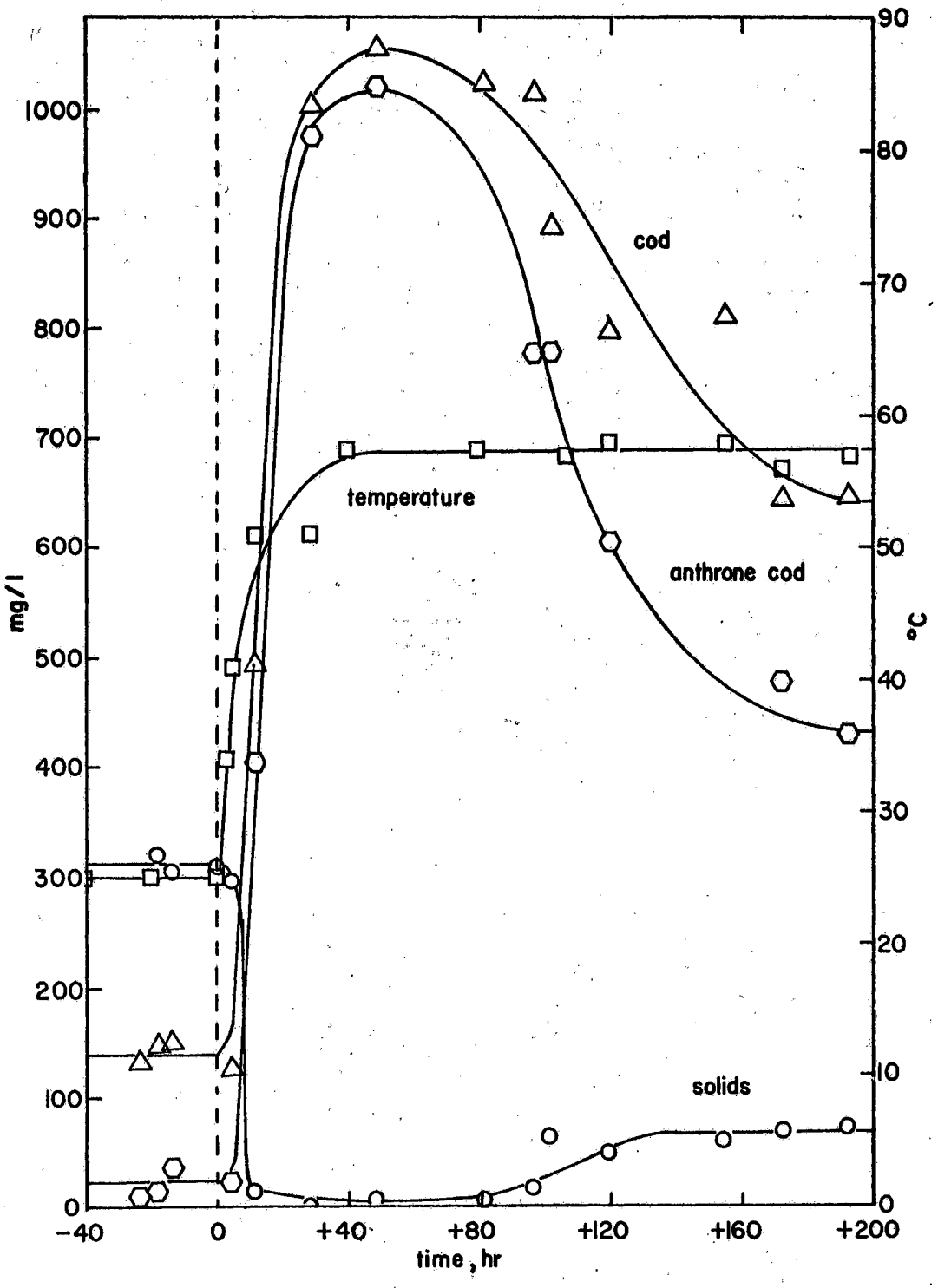

Figure 72 - Response of a system shock loaded from 25°C to 57.5°C (detention 4 hours).

TABLE XII

CHANGES IN EFFLUENT COD AND BIOLOGICAL SOLIDS IN RESPONSE TO TEMPERATURE SHOCK LOADS

	Detention 8 hours					Detention 4 hours				
Initial Steady State Temperature, °C	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0
Final Steady State Temperature, °C	8.0	17.5	36.0	47.0	57.5	8.0	17.5	36.0	47.0	57.5
Time to reach the New Temperature, hr	11	6	40	29	50	11	6	40	29	50
Initial Steady State COD, mg/l	100	60	72	100	66	72	45	64	84	138
Maximum Transient State COD, mg/l	-	186	-	924	1062	-	372	-	1068	1056
Time to reach the Transient State COD, hr	-	32	-	35	62	-	24	-	53	55
Minimum Transient State Solids, mg/l	-	414	420	93	8	-	240	476	12	3
Time to reach the Minimum Solids, hr	-	20	110	35	50	-	30	11	30	50
Final Steady State COD, mg/l	930	48	-	135	450	1020	624	80	132	648
Time to reach the Final Steady State COD, hr	64	100	-	54	120	172	120	130	112	170
Final Steady State Solids, mg/l	24	594	-	306	180	6	390	426	318	66
Time to reach the Final Steady State Solids, hr	32	160	-	70	130	34	70	130	110	140
Maximum Intermediates released, mg/l	300	169	-	882	420	300	364	-	270	216
Time to attain Maximum Intermediates, hr	72	32	-	35	10	10	20	-	20	193

recover, but the one at eight hour detention time recovered completely. Also, at both detention times during the temperature change from 25 to 36°C there was no significant peak in the transient response for COD. As the magnitude of the temperature shock increased to 47 and 57.5°C it is to be noted that the transient peak value of COD reached approximately the concentration of COD in the influent, indicating complete loss of treatment efficiency. However, at the post-shock temperature of 47°C both systems (four and eight-hour detention time) acclimated, and purification capacity was restored. At 57.5°C the recovery was only partial. The mixed liquor biological solids concentration in the systems during the transient state decreased progressively as the severity of the shock increased. The magnitude of the post-shock steady state COD also increased with the severity of the temperature shock load; also, the effluent COD was higher for the experiment run at the four-hour detention time when compared to that at the eight-hour detention time (except at 36°C). The biological solids level at the new steady state also decreased as the shock temperature was increased. The amount of metabolic intermediates and/or end products as shown by the difference between COD and anthrone COD was a maximum during the temperature shock from 25 to 47°C at the eight-hour detention time, and the maximum value occurred at thirty-five hours (4.4 detention times) after the shock.

Pre-shock and post-shock parameters for the various

experiments are compared in Table XIII. At the eight-hour detention time the post-shock steady state performance in the range 17.5-47°C lies in an acceptable range of effluent COD level. The system could not establish an acceptable final steady state performance at 8°C or 57.5°C. At 57.5°C the results were somewhat better than at 8°C, but solids level and COD removal efficiency did not return to the pre-shock level during the experimental period. Minimim protein content of the sludge occurred at 17.5°C (37 per cent), and maximum at 25 and 36°C (51 per cent). However, it is to be noted that at 25°C the average protein content varied from 40 to 51 per cent. Regarding the carbohydrate content, the only significant change was at the 47°C shock temperature (17 to 7.5 per cent).

At the higher growth rate (four-hour detention time) satisfactory new steady state performance was attained in the temperature range 25 to 47°C. The effluent COD levels at 17.5 and 57.5°C were over 600 mg/l, and at 8°C the system was essentially washed out. The COD removal efficiency decreased with the severity of the shock load. The decrease was generally faster at the four-hour detention time as compared to the eight-hour detention time. Concerning protein content, an increase in temperature caused an increase in protein content of sludge, and a decrease was registered for a decrease in temperature. Increase in temperature caused significant decrease in carbohydrate content. At either detention time it would appear that higher operating

TABLE XIII

STEADY STATE PARAMETERS IN ALL EXPERIMENTS BEFORE AND AFTER APPLYING TEMPERATURE SHOCK LOADS

Initial Steady State (25°C)						Final Steady State						
Solids mg/l	COD* mg/l	COD* Removal Efficiency %	Anthrone COD* mg/l	Protein %**	Carbohydrates %**	Reactor Temperature °C	Solids mg/l	COD* mg/l	COD* Removal Efficiency %	Anthrone COD* mg/l	Protein %**	Carbohydrates %**
Detention 8 hours												
372	104	90	50	-	-	**8.0**	24	930	12	630	-	-
540	60	94	18	40	21	**17.5**	594	48	96	18	37	20
488	72	93	22	51	12	**36.0**	480	32	96	6	51	12
510	99	91	54	42	17	**47.0**	306	135	87	18	45	7.5
488	66	94	18	-	-	**57.5**	180	450	58	96	-	-
Detention 4 hours												
558	74	93	24	-	-	**8.0**	6	1020	4	894	-	-
516	45	96	6	45	18	**17.5**	390	624	42	597	40	18
576	64	94	16	43	13	**36.0**	426	80	92	16	55	9.3
516	84	92	30	42	20	**47.0**	318	132	88	30	50	9
312	138	87	24	-	-	**57.5**	66	648	39	432	-	-

*COD refers to Effluent COD

**Protein and Carbohydrates Expressed as % of Sludge

temperatures can be expected to cause a decrease in carbohydrate content of the sludge.

Table XIV gives the yields at the various steady states in the several temperature shock loading experiments. At both detention times the yield of cells is generally lower at higher temperatures. The variation in yield of carbohydrates was greater than in the protein. Beginning with the 17.5°C temperature, the new steady state parameters, i.e., the yield of cells, protein and carbohydrate, decreased progressivly as the temperature was increased.

Changes in microbial morphology were noted at the various temperatures employed. The primary morphological change which was noted was that the short thick rod forms which appeared to predominate at the low temperatures shifted to thin elongated forms at the higher temperatures. At 47°C the cells upon approaching a new steady state elaborated a soluble greenish-grey pigment, which disappeared after they attained the new steady state. The observed changes in morphology and pigment production may be the result of changes in microbial predominance.

TABLE XIV

YIELD OF CELLS, PROTEIN AND CARBOHYDRATE AT STEADY STATES BEFORE AND AFTER TEMPERATURE SHOCK LOADING

Initial Steady State				Final Steady State			
Reactor Temperature °C	Yield of Cells %	Yield of Protein %	Yield of Carbo-hydrates %	Reactor Temperature °C	Yield of Cells %	Yield of Protein %	Yield of Carbo-hydrates %
Detention 8 hours							
25.0	39	-	-	8.0	18	-	-
25.0	54	22	11	17.5	59	22	12
25.0	49	25	5.9	36.0	47	24	5.6
25.0	53	22	9	47.0	33	15	2.5
25.0	49	-	-	57.5	30	-	-
Detention 4 hours							
25.0	57	-	-	8.0	15	-	-
25.0	51	23	9.2	17.5	87	35	16
25.0	58	25	7.5	36.0	44	24	4.1
25.0	53	22	10.6	47.0	34	17	1.3
25.0	34	-	-	57.5	16	-	-

CHAPTER VI

DISCUSSION

Much research in the waste water treatment field in recent years has been devoted to the delineation of basic mechanisms underlying the various biological processes. It is becoming more and more apparent that more enlightened design and reliable operation of biological treatment processes depend upon a better understanding of the biochemical relationships governing the response of natural populations. Most of the attention has been given to steady state operation, but there have been some studies aimed at determining the response to changes in the external environment. There have been various studies in the basic microbiological field dealing with depiction of the transient responses to some types of shock loadings studied in the present investigation; however, they were accomplished for the most part without regard to parameters of importance to the pollution control field. Furthermore, it is difficult to extrapolate the results of studies with pure cultures to results which one might obtain using heterogeneous populations. The studies undertaken in the present investigation were intended to throw some light on the basic aspects of the biochemical response, but more importantly, by use of heterogeneous

populations, the work was intended to be more applicable to the waste water treatment field.

A. Hydraulic Shock Loads

Hydraulic shock loads were studied under two distinct conditions, i.e., conditions of constant influent organic concentration, and conditions of constant daily organic loading. Under conditions of constant concentration the amount of organic matter entering the reactor in a given time increased as the dilution rate increased and decreased for shock loads involving a decrease in dilution rate. Under constant loading conditions the amount of organic matter entering the reactor per unit time remained constant regardless of the change in dilution rate. It was desirable to study both types of conditions, since both types of environmental change may be imposed upon systems in the field, and from a basic standpoint it was desirable to assess possible relationships between these two types of environmental change. In all experiments the steady state detention time before imposing the shock load was eight hours, and an inflow concentration of 1000 mg/l glucose was maintained. At the eight-hour detention time this glucose feeding concentration corresponded to an organic loading of 198 lbs of COD/1000 cu ft of aerator volume per day. Under both conditions of shock loading the flow rate was changed over a wide range (25 to 350 per cent). Under conditions of constant concentration the change in flow rate led to changes in organic loadings from 49.5 to 693 lbs of COD/

1000 cu ft of aerator capacity per day, whereas under constant loading conditions the reactor loading remained constant at 198 lbs COD/day per 1000 cu ft of aerator capacity, and glucose concentration was appropriately varied from 4000 to 286 mg/l.

Under conditions of constant concentration shock loads involving an increase in detention time (decrease in dilution rate) did not cause serious malfunction of the system with respect to effluent COD concentration, as can be seen from Figures 2, 4, and 6. However, these shock loads did cause some fluctuation in the level of biological solids. These results indicate that shock loads of this type can be successfully handled by the biological population. It might be argued that such a result should have been anticipated, and indeed it was. On the other hand one could not say, without experimental evidence, that the forcible change of growth rate would not exert a deleterious effect severe enough to dilute out cells. However, based upon the observations made during these experiments, it can be said that any possible deleterious effects due to the forcible metabolic "slowing down" period was more than made up for by the increased detention time, and disruption of system efficiency did not ensue.

Regarding changes in sludge composition during these shock loadings, there appeared to be an increase in the RNA level as the dilution rate was decreased (see Figure 3). The increase in RNA does not appear to be very significant,

but it is important to note that Herbert's results indicate that the reverse should have occurred, i.e., RNA content increases as dilution rate is increased (22). Herbert's findings seem reasonable, since at higher dilution rates (growth rates) a higher complement of RNA is necessary to accommodate the faster rate of cell synthesis. However, in the present study it must be remembered that heterogeneous populations were employed, and changes in predominance were noted as the dilution rate was decreased. This was particularly evident for the shock load leading to a detention time of thirty-two hours; a distinct change in color of the reaction liquor was noted. Since the level of RNA may vary with the bacterial species (22), it is possible that cells with a normally higher RNA level predominated at the higher detention time. It is interesting to note that Cassel, et al. (28) have also noted changes in microbial predominance with different dilution rates. Changes in protein and carbohydrate content during the shock load leading to detention times of sixteen hours (Figure 5) and 10.67 hours (Figure 7) indicated that there was a slight rise in protein content and, at the sixteen-hour detention time, a significant decrease in the carbohydrate content.

The results for hydraulic shock loadings under constant concentration conditions leading to increased dilution rates or decreased detention times were shown in Figures 8 to 17. It is important to note that the system successfully responded to a fifty per cent increase in flow rate (detention

time eight to 5.33 hours) without a significant rise in COD of the effluent. This shock load led to a change in organic loading from 198 to 297 lbs of COD/1000 cu ft of aerator capacity per day. Even at a 100 per cent hydraulic and organic overload (see Figure 10) there was very little disruption of COD removal efficiency in the transient state. However, at this shock loading level a response which was found to be fairly characteristic of all succeeding shock loads (hydraulic) at lower detention times in this series at constant concentration became evident. In response to the increase in flow rate, the solids concentration decreased slightly and COD concentration increased. Following this, the solids level began to increase, and caused a concomitant decrease in the concentration of the effluent COD. At this shock loading level the leakage of COD was not due to the leakage of glucose, but was attributable to metabolic intermediates and/or end products.

For the more severe hydraulic shock loadings (see Figures 12, 14 and 16), the same general pattern of solids dilute-out and recovery and COD leakage and recovery were experienced. However, as the dilution rate increased, solids recovery and recovery of COD removal efficiency became progressively less complete. At the most severe shock loading (Figure 16) there was a smooth transition to the new steady state, i.e., there was no dip in the biological solids curve followed by a rise in solids concentration through the new steady state level. The fact that the

new steady state solids concentration was lower than that before the shock at dilution rates greater than 0.313 $hour^{-1}$ indicates that at these dilution rates the system was approaching dilute-out. Values of μ_{max} and K_s were not determined in these studies; however, it is interesting to note that Gaudy, Ramanathan and Rao (99) observed that cell dilute-out was initiated in this range of dilution rates for heterogeneous populations grown on glucose at 1000 mg/l. It is of interest to compare the present results with those of Krishnan, who studied quantitative shock loadings. He observed (20) that a completely mixed system operating at a detention time of eight hours was capable of accommodating, without serious impairment of system efficiency, an organic overload of 200 per cent, i.e., an increase in substrate concentration from 1000 mg/l to 3000 mg/l. In the current studies it is seen that a hydraulic overload of 100 per cent, i.e., a decrease in detention time from eight hours to four hours, can be accommodated without serious impairment of system efficiency. Thus, it may be stated that completely mixed systems exhibit a considerable degree of versatility with respect to biochemical efficiency of substrate removal. It is emphasized that these studies were concerned solely with the biochemical efficiency of the system; since sludge recycle was not practised and the systems were entirely dispersed, the above statements regarding the magnitude of environmental change which can be accommodated do not necessarily apply to over-all system

efficiency, which would include sludge settleability.

The data on cell composition before, during, and after the hydraulic shock loadings leading to an increase in dilution rate were shown in Figures 9, 11, 13, 15, and 17. From these figures it may be discerned that the general trend as dilution rate increased was a decrease in the protein content of the sludge and an increase in the carbohydrate content. From these results it would appear that the cells cannot begin to replicate immediately at the high growth rate equal to the imposed dilution rate. This is evident from immediate decrease in cell concentration, and this would appear to be substantiated by the fact that the protein content of the cells in the unit at any time immediately after the shock decreased. However, the fact that the carbohydrate content in the system rose indicates that the synthetic capability of the cells for making components other than protein is not as seriously impaired. It should be noted that the increase in percent carbohydrate of the cells comes about not simply because the protein is diluted out; for those experiments for which the absolute concentration of carbohydrate in the cells is shown, e.g., Figures 8, 10, and 12, it is seen that the carbohydrate concentration increased. The RNA content of the sludge was assessed for the most severe shock loading (dilution rate 0.437 hour^{-1}) shown in Figure 17; the RNA content increased throughout the transient response. This result was in agreement with the findings of Herbert, who used a pure

culture of Aerobacter aerogenes (22). This result seems highly plausible, since even if a change in predominance occurred, one would expect faster growing cells to contain a higher complement of RNA.

Response to shock loadings under constant loading conditions for changes leading to decreased dilution rates were shown in Figures 18, 21, and 23. It will be remembered that under these conditions the incoming substrate concentration was increased as the detention time was increased in order to keep the loading constant. If the response of the system were governed by the magnitude or rate of organic loading, one would expect that a doubling or quadrupling of detention time would accommodate a doubling or quadrupling of substrate concentration in the inflow. While this surmise seems plausible for steady state operation, there is no guarantee that there would be a smooth transition during which no substrate would be lost in the effluent, nor can one predict the magnitude of the complementary changes in detention time and substrate concentration which could be assimilated by the system without a severely deleterious transient response. The results shown in Figure 18 clearly indicate that an increase in detention time from eight hours to thirty-two hours accompanied by an increase in substrate concentration from 1000 mg/l to 4000 mg/l, could not be smoothly effected without disruption of substrate removal efficiency. The maximum decrease in COD removal efficiency during the transient state was 8 per cent. The system

returned to its previous steady state level of COD removal efficiency in slightly over six detention times. It is seen in Figure 21 that when the detention time was doubled and the influent substrate concentration was doubled, there was a smooth transition without any loss of substrate removal efficiency. Comparing these responses under constant loading conditions with those responses leading to increased detention times for constant concentration conditions, it may be seen that while rather severe environmental changes can be imposed without very serious deterioration of substrate removal efficiency, the constant loading condition does appear to lead to a somewhat more deleterious transient response. This would appear to arise primarily because of the fact that under constant loading conditions the system actually receives a quantitative shock loading when the detention time is increased. These results have emphasized the fact that the constant loading parameter, i.e., equivalence of organic concentration and detention time, so often relied upon for the design of treatment plants, is after all an artificial parameter established for use in steady state conditions and does have limits of application with regard to operational considerations such as shock loadings.

Changes in sludge composition during a shock under constant loading conditions leading to increases in detention time were shown in Figures 19, 22, and 24. The general trend under these conditions indicates a decrease in protein

and RNA and an increase in carbohydrate and DNA in response to the shock loading. The reverse trend was observed for the same hydraulic shock loads under constant concentration conditions.

The results for hydraulic shock loadings leading to an increase in dilution rate and concomitant decrease in concentration of the carbon source (constant loading conditions) were shown in Figures 25 to 30. It was seen that, as would be expected, the biological solids concentration decreased in response to this type of shock load. It is important to note that there was at no time a severe transient response involving an increase in effluent COD. It would appear, therefore, that during the period of cell dilute-out in the transient state, there was sufficient time for the population to adjust to the new growth rate or sufficient time for selection of cells which could exist in the unit at the higher growth rate. The types of shock loads applied during this series of experiments are those which might be expected as a result of storm water runoff, and it would appear from these results that such shock loads cannot be expected to decrease biochemical efficiency. However, it is important to emphasize again that such shock loads may have serious effects upon sludge settleability.

Changes in sludge composition during two of the shock loads of this series were shown in Figures 27 and 29. The results indicate a decrease in protein content, and an increase in carbohydrate content of the sludge. The same

general trend of changes in protein and carbohydrate content were observed for shock loadings of increased dilution rates under constant concentration conditions. The trends seem to indicate that at higher steady state growth rates the cells have a tendency to store carbohydrate or that the higher dilution rates tend to select cells with higher carbohydrate content.

Figure 31 showing the changes in yield and COD removal efficiency clearly indicates that a change in flow rate at constant loading conditions causes more variation in yield than a change under constant concentration conditions. From this it appears that the change in yield of cells is dependent more on the influent organic concentration, and less on the daily organic loading. Under constant loading conditions change in yield tends to increase when the influent organic concentration tends to decrease, and vice versa. However, for the system shocks resulting in a dilution rate in the kinetic "dilute-out" region, the change in yield decreases, i.e., the yield after the shock approaches the value it had before the shock. The fact that yield did not stay constant even at pre-shock steady state during these constant loading experiments is clearly established. This finding is in opposition to the theory of Servizi and Bogan (100), which stated that there is a constant proportionality between yield and free energy of oxidation of the substrate. It would appear that in heterogeneous systems yield may be dependent upon several factors, including the

predominating species, the detention time, and, at least during hydraulic shock loading conditions, on the influent organic concentration. The importance of the predominating species or organisms in the determination of yield was demonstrated by Rao and Gaudy (101). The data on hydraulic shock loads also indicated some changes in predominance at extreme variations in dilution rates, and hence the predominating species may be involved in the determination of yield in heterogeneous systems. The negative change in COD removal efficiency under constant concentration conditions at the dilution rates higher than 0.25 $hour^{-1}$ is believed to be due to the fact that at these dilution rates the systems were obeying the kinetic dilute-out to be expected in accordance with steady state theory (99).

B. pH Shock Loads

All pH shock loading studies were conducted at a detention time of eight hours. The change in pH was effected by varying the phosphate compounds in the feed. However, for all experiments the COD/P ratio was maintained at 3.4/1. In all cases the pH at the initial steady state, i.e., before applying the shock loading, was approximately 6.5.

The results for shock loadings which increased the pH were shown in Figures 32 to 36 for systems in which sludge recycle was not employed. This type of shock loading, at the levels of pH investigated, caused no deleterious effects with respect to effluent COD and anthrone COD. However, it is seen that the system was not unaffected by these

pH changes, since the biological solids concentration was subject to rather wide fluctuation after the shock. Changes in sludge composition at these shock loading levels did not appear to be significant, although there was a noticeable reduction in protein content and the carbohydrate content increased somewhat. Results for the system in which sludge recirculation was practised were shown in Figures 34 and 35. It is interesting to note that the pH of the feed solution was the same as that for the experiment shown in Figures 32 and 33, but the resulting reactor pH was significantly lower than that observed in the once-through system, showing the effect of higher solids concentration in the unit in which sludge recycle was practised. The major difference in response between these two experiments is the transient rise in solids concentration, and the higher level of solids maintained at the new steady state. The higher solids level is not a result of changes in the concentration or flow rate of the recycled sludge, and there is no readily apparent explanation for the increase in solids concentration. There was no readily observable change in microbial predominance. Comparison of the two results does indicate that COD removal was somewhat improved when cell recycle was employed.

The results of shock loads leading to acid pH were shown in Figures 38 to 52. These shock loadings were studied in greater detail, since they are in all probability the most common ones encountered in the field, and, due to the normally higher production of carbon dioxide in aerobic

systems, it would certainly seem that activated sludge processes are less poised to receive acid shock loads than alkaline shock loads. Also, the operational problems encountered with the alkaline shock, i.e., precipitation of inorganic salts militated against their study at very high pH values. Since a considerable number of acid pH shock loads were investigated, it seems appropriate first to discuss the results for those cases wherein sludge recycle was not practised. Responses with respect to solids concentration and effluent COD for these cases were shown in Figures 38, 40, 41, 47, and 49, and cover shocks leading to pH from 6.15 to 3.00. In all these cases the system recovered completely from the shock loadings. The general trend of response in the transient state was a decrease in solids and concomitant increase in COD of the effluent, followed by a recovery of the solids concentration and a decrease in COD of the effluent and eventual recovery of COD removal efficiency. The results of microscopic observations made during the period of decreasing and increasing solids concentration during these experiments (see Figures 53 through 56) indicate that extreme changes in microbial predominance occurred. It was observed that before imposing the shock loadings, the predominant microbial forms present were rod-shaped bacteria and many protozoa were present; throughout the transient state, filamentous forms gained predominance. It is interesting to note that Slyter, et al. (46), in their studies on the effect of pH on population dynamics

and fermentation in a simulated rumen ecosystem, observed that protozoa were present in low concentrations at acid pH values. Also Rogers and Wilson (63), in their studies on the decomposition of sewage in acid mine drainage water, observed that in the pH range 5 to 7 the microbial population consisted mostly of bacteria, and in the pH range 3.5 to 5, yeasts predominated. From the present studies and results of the studies cited above, it seems evident that a change in pH imposes an extremely selective pressure upon biological systems. It is interesting to note that sludge yield in the steady state at lower pH levels is, in general significantly higher than the steady state yield which was observed at the neutral pH prior to the shock. Some investigators have attempted to relate sludge yield to waste water COD, claiming that for certain types of compounds, for example, carbohydrates, the sludge yield was a constant fraction of the COD of the waste. From these results it can be seen that such generalization is not possible, since a change in pH with no change in COD of the waste caused considerable change in sludge yield. In regard to the leakage of COD in the effluent, it seems apparent from the results of shock loadings leading to pH values of 6.15 and 5.75 that such changes in pH could be accommodated with relatively little disturbance of the system. For the shock leading to the pH value of 5.75 there was no leakage of COD; for the milder shock (pH 6.15) there was some leakage of COD. However, this result would appear to be somewhat

atypical in that the COD response was somewhat delayed. It seems reasonable to conclude that a change in pH from neutrality, i.e., 6.5, to pH 5.75 can be adequately accommodated from the standpoint of biochemical efficiency of the system. Shock loads in between those leading to pH values of 5.75 and 3.50 could not be made because of poor buffering action of phosphate salts in this range of pH, and it could not be determined from this study at which pH severe disruption may begin to ensue. However, it is apparent from the results obtained at pH levels of 3.5 and below that such shock loadings can be expected to cause severe disruption of the system. However, it is also apparent that these once-through systems could recover from shocks down to pH 3.0. At this range of pH the point of maximum COD in the effluent (and minimum mixed liquor suspended solids) occurred in approximately three to four detention periods after administering the shock. Recovery of COD removal efficiency took place within ten to fifteen detention times.

The biological solids and COD responses which were shown in Figures 43 and 45 for which sludge recirculation was practised, together with the response shown in Figure 47, offer a convenient means for comparing the effect of biological solids level on the response to comparable pH shock loadings. The steady state biological solids concentration in the reactor for the experiment shown in Figure 43 was maintained at approximately 2200 mg/l before the

shock by recycle of a large portion of the sludge which settled in the clarifier. The biological solids concentration decreased rather severely to approximately 900 mg/l; however, the COD removal efficiency was for all practical purposes undisturbed. One of the primary reasons for decrease in the solids was the progressive disruption of flocculating and settling characteristics of the sludge after administering the shock loading. Microscopic observations indicated that as the solids concentration level dropped, a larger proportion of the population consisted of filamentous organisms; however, throughout the experiment bacteria predominated. For the results which were shown in Figure 45 the steady state solids level before the shock was approximately 600 mg/l. This solids level was maintained by recycling a smaller portion of the solids. Furthermore, an attempt was made to maintain the recycle solids input constant by centrifuging the supernatant and making up the recycled solids to the desired constituency. The recycle volume was 33.33 per cent of the inflow, and on the average, the biological solids concentration in the recycled sludge was 1000 ± 100 mg/l. The general response to this shock load was similar to that observed for comparable shock loading without recirculation (see Figure 47); however, the maintenance of a higher initial steady state solids concentration prevented the nearly complete dilute-out of cells experienced in the once-through system, and resulted in less leakage of COD in the effluent (600 mg/l

compared to 900 mg/l). These experiments provide a definite indication of the beneficial effects of maintaining higher biological solids concentration for providing greater ability to accommodate pH shock loadings, even though sludge recycle has a tendency to suppress changes in predominance. Although one might expect that the best way to hasten recovery from an acid shock would be to seek ways and means to hasten a change in predominance to organisms which were best suited to the environment, it would appear that cells retained from the previous steady state can acclimate to the new pH condition and make a positive contribution to the recovery of system efficiency.

Changes resulting in pH values below 3.0 (see Figure 52, pH 2.7) cannot be accommodated, and in the present studies led to nearly complete wash-out of the system. It is, however, interesting to note that the biological solids did not completely wash out, and the substrate concentration in the reactor did not become as high as the influent concentration, indicating that some organisms were growing, and it seems entirely possible that with prolonged acclimation to this pH condition the system may have recovered.

Changes in sludge composition during the acid pH shock loading studies were shown in Figures 39, 42, 44, 46, 48, and 50. The following trends seem apparent during the transient and the new steady state. In general, the protein content of the cells decreased during the transient, and remained low in the new steady state. On the other hand,

the carbohydrate content increased during the transient and remained at a higher level during the new steady state. The RNA and DNA contents of the cells also increased during the transient, and the RNA content remained slightly higher than in the initial steady state after attaining the new steady state condition; after increasing in the transient state, the DNA content decreased to a new lower level. It is emphasized that the response and recovery pattern for the shock loads indicated that the population changed from one consisting primarily of bacteria and protozoa to one consisting mainly of filamentous organisms. Protein content of the sludge before the shock loads varied from 51 to 65 per cent, which compares well with values reported in the literature for bacterial populations. The protein content in the new steady state resulting from the pH shock load varied from 15 to 39 per cent, a range more consistent with the protein content of 10 to 25 per cent reported in the literature for fungi (102). A reliable range of carbohydrate content of the fungi could not be found in the literature, but from the present results it would appear that the carbohydrate content of the fungi can reach higher values than those reported for bacteria under steady state conditions. The changes in RNA and DNA content during the transient, i.e., the increasing trend, seems predictable on the basis of the observed changes in predominance during the shock loading. It may be reasoned that as the bacterial cells were diluting out of the system and a new population

consisting predominantly of fungi was growing up, under conditions of high substrate concentration wherein their growth rate was not precisely controlled by dilution rate, larger amounts of materials needed for replication may be produced.

C. Temperature Shock Loads

It should be recalled that studies on temperature shock loading were conducted at four-hour and eight-hour detention times, and at either detention time the rapidity of the change in temperature for any specific temperature shock was approximately the same. The results for shocks leading to temperatures below the initial steady state temperature were shown in Figures 57 through 62. The most severe shock applied was one which led to a new steady state temperature of 8°C, and it is seen by comparing Figures 57 and 58 (eight-hour and four-hour detention times, respectively) that the detention time in the unit or the growth rate did not play a major role in determining the course of the response, i.e., the system efficiency was disrupted with nearly the same rapidity and to nearly the same extent at the eight-hour detention time as at the four-hour detention time. The rather poor response at this extreme cool temperature may be due to intracellular damage to some cells and death of other cells. According to Kavanau (93), "the sugar transport mechanisms are affected by conformational changes in the protein carriers due to their hyperfolding caused by the increase in the number of intermolec-

ular hydrogen bonds." Kavanau's hypothesis could explain the inactivity of the system as being due to partial shielding of active sites, thereby affecting the rate of enzymic reaction. Strange and Dark (78)found that when certain gram negative organisms which were growing in the exponential phase in the temperature range 30-40°C were suspended in a diluent such as water or buffer and rapidly cooled to 0-5°C, a large portion of the organisms were killed and they released nucleotides, amino acids, and ATP into the medium. Analyses for such intracellular components in the medium were not made during the present studies; however, it is noted that at the four-hour detention time acetic acid was found in the medium. In any event, it may be surmised from the results shown in Figures 57 and 58 that the system cannot be expected to exhibit recovery from a severe cold shock within a reasonable period.

Regarding the milder cold shock, i.e., 25 to 17.5°C, shown in Figures 59 and 61, it may be discerned that the longer detention time afforded greater resistance to the environmental change.

Due to the fact that biological solids nearly diluted out at the severe cold shock, analyses for cell composition were not made; however, at the milder cold shock, analyses for protein, carbohydrate, RNA and DNA were made. The results at the eight-hour and four-hour detention periods were shown in Figures 60 and 62 respectively. The changes in sludge composition at both detention times exhibit the

same general trend; during the period of cell dilute-out there was a slight rise in protein and RNA content of the sludge and a slight decrease in cellular carbohydrates; the DNA content remained approximately the same throughout the transient. The concurrent increase in RNA and protein could be indicative of unrestricted growth (i.e., in high substrate concentration) of cells which were capable of growing at the lower temperature. While incapacitated cells were diluting out of the system, a shift in species predominance could have been occurring which was reflected in higher levels of RNA and protein of these more rapidly growing cells. It is noted, however, that no changes in predominance could be detected by microscopic observations.

The responses of systems to an increase in temperature were shown in Figures 63 to 72. The typical pattern for all responses involves a depression and recovery of the biological solids curve and an increase in effluent COD followed by recovery of COD removal efficiency. Microscopic observations made during the course of these responses indicated that changes in predominance occurred during the period of cell dilute-out and recovery. These were particularly evident at the 47 and 57.5^{o}C temperature. At the initial steady state temperatures the predominating forms were short, thick rods; these began to dilute out of the system during the depression in biological solids concentration and were gradually replaced by thin elongated cells which came into greater and greater predominance as the

biological solids concentration recovered. Thus the shape of the response curves for solids and COD can be attributed to the effect of a shift in predominance.

Sludge protein, carbohydrate, RNA and DNA were determined for shock loadings at the 36 and 47°C level, and the results were presented in Figures 64, 66, 68, and 70. The general trend appears to indicate that at the faster growth rate (four-hour detention time) as the temperature was increased, the percent protein increased and the percent carbohydrate and RNA decreased. It will also be recalled that cell yield decreased as the temperature was increased. There is insufficient information concerning the effects of elevated temperatures on cellular components to allow interpretation of these results. The coupling of an increase in protein content with a decrease in RNA content would appear to be somewhat inconsistent unless elevated temperatures affect these components differently.

In general, it may be tentatively concluded that a system operating initially at a temperature of 25°C can more readily absorb shock loadings of higher temperature than colder temperatures. Also, the detention time appears to play a role in determining the course of the response. The leakage of effluent COD during the transient condition was lower, and the system recovered faster at the eight-hour detention time than at the four-hour detention time. In analyzing these results it is necessary to stress once again that in these studies it was the biochemical effi-

ciency of the response which was determined, i.e., the effect of the shock on sludge settleability was not the subject of investigation. The only other study reported in the literature dealing with changes in temperature of continuously cultured heterogeneous populations is the study of Dougherty and McNary (88). They did not give the detention time in the experimental apparatus they employed; however, from the data they do provide, the best approximation for the detention time is eighteen hours. Also, in their studies they employed solids recycle and the change in temperature was considerably more gradual than in the present studies. They found that when the temperature of the culture was raised by 10°C (in the range of study 21-46°C), the effluent became turbid and the effluent BOD increased; after a few days the sludge "acclimated" and the effluent BOD returned to its previous concentration. Since in the present studies a rise in temperature from 25 to 36°C did not cause any significant increase in the filtrate COD, i.e., no decrease in biochemical efficiency, it may be surmised that the rise in effluent BOD observed by Dougherty and McNary was primarily due to a decrease in the flocculation and settling abilities of the organisms. It is significant to note that they, too, observed a change in predominance during the period in which the temperature was changed.

D. Variation in Steady State Parameters at Constant Detention Time

It will be recalled that for most of the shock loading studies, the initial steady state before applying the shock was attained at a dilution rate of 0.125 $hour^{-1}$ (eight-hour detention time) and this steady state prior to applying any shock was always attained by starting the unit on fresh seed of heterogeneous population from the municipal sewage treatment plant in Stillwater, Oklahoma. Thus, the data obtained before applying the shock is valuable from the standpoint of determining the degree of variation under conditions for which the only variable was the initial heterogeneous population; these data are summarized in Table XV. Each value shown in the table represents an average obtained from analyses of three to five samples. It is seen that even under constant operational conditions (temperature 25 $\pm$ 1°C, pH 6.55 $\pm$ 0.15, dilution rate 0.125 $\pm$ 0.004 $hour^{-1}$, or detention time 8 hours $\pm$ 0.25 hour; constant composition of the growth medium and constant air flow rate) there was considerable variation in the so-called "steady state" parameters. It is interesting to note that Rao and Gaudy (101) working with batch operated activated sludge, also observed considerable variation in cell yield for heterogeneous populations. Krishnan (20), during the course of conducting continuous flow studies pertinent to quantitative shock loads, also noted considerable variation in the steady state cell yield prior to applying the shock. It seems apparent from Table XV that the procedure employed in the present study, i.e., determination of steady state condi-

TABLE XV

DATA FOR "STEADY STATE" PARAMETERS BASED ON ALL EXPERIMENTS AT 8-HOUR DETENTION TIME AND 1000 mg/l GLUCOSE IN THE INFLUENT

Figure Number 1	Solids mg/l 2	Effluent COD mg/l 3	Yield of Cells % 4	COD Removal % 5	Protein in Sludge % 6	Carbohydrate in Sludge % 7	RNA in Sludge % 8	DNA in Sludge % 9	Anthrone COD mg/l 10
4, 5	510	80	52	92	50	21	-	-	32
6, 7	460	94	48	91	38	12	-	-	16
8, 9	580	52	58	95	57	15	-	-	38
10, 11	436	80	44	92	60	23	-	-	40
12, 13	532	75	54	93	54	19	-	-	34
14, 15	536	138	58	87	53	19	-	-	22
16, 17	540	80	55	92	49	13	18	-	12
18, 19	412	112	43	90	47	25	28	3.5	76
21, 22	420	71	42	93	51	5.7	-	-	10
23, 24	563	54	56	95	53	17	-	-	25
25	536	52	53	95	-	-	-	-	24
26, 27	380	124	41	88	53	12	-	-	48
28, 29	304	148	33	86	59	12	-	-	54
30	344	160	38	85	-	-	-	-	68
32, 33	464	100	48	91	30	7.5	-	8.0	4
36, 37	492	52	49	95	35	7.5	14	8.0	20
38, 39	480	100	50	91	-	-	9	3.2	36
40	530	100	55	91	-	-	-	-	20
41, 42	375	225	45	79	56	15	-	3.6	-
47, 48	432	66	43	94	47	19	8	2.5	16
49, 50	450	144	49	86	67	13	13	4.0	10
52	609	93	63	91	-	-	-	-	12
57	372	104	39	90	-	-	-	-	50
59, 60	540	60	54	94	39	21	9.8	3.5	18
63, 64	488	72	49	93	51	12	9	2.5	22
67, 68	510	99	53	91	42	17	16	3.5	54
71	488	66	49	94	-	-	-	-	18
Maximum Value	609	225	63	95	67	25	28	8.0	76
Mean Value	473	94	49	91	50	15	13.9	4.2	30
Minimum Value	304	52	33	79	30	5.7	8	2.5	4
Percent Variation from Mean Value	+29	+139	+29	+4.4	+34	+67	+101	+90	+153
	-36	-45	-33	-13	-40	-62	-42	-40	-87

tions prior to each individual shock loading experiment, was an extremely useful one without which the responses could not have been analyzed. The data shown in Table XV also demonstrate that when dealing with the steady state one cannot expect such an important "system constant" as sludge yield to be a precise constant, and that it is extremely important to consider the range of variation in the so-called system constants in designing waste water treatment plants.

CHAPTER VII

SUMMARY AND CONCLUSIONS

A. Hydraulic Shock Loads

1. Under constant concentration conditions an increase in flow rate resulting in dilution rates greater than 0.25 $hour^{-1}$ (detention times less than four hours) gives a deleterious transient state response as indicated by a decrease in biological solids and increase in effluent COD. On the other hand, under constant organic loading conditions, a decrease in flow rate resulting in dilution rates lower than 0.062 $hour^{-1}$ (detention times greater than sixteen hours) causes a severe transient state response. Thus a steady state system operating at a dilution rate of 0.125 $hour^{-1}$ (eight-hour detention time) can accommodate no greater than a 100 per cent increase in flow rate under constant concentration conditions or a 50 per cent decrease in flow rate under constant loading conditions if a severe transient state response is to be avoided. Decrease in flow rate under constant concentration conditions and increase in flow rate under constant loading conditions do not result in a response as severe as seen during the opposite conditions of shock loading.

2. Based on steady state values before and after shock loading, it was seen that shock loading under constant concentration conditions causes significant reduction in COD removal efficiency at dilution rates in excess of approximately 0.25 $hour^{-1}$ (less than four-hour detention time). However, under constant loading conditions the changes in COD removal efficiency are less significant at increased flow rates, compared to the constant concentration conditions.

3. Hydraulic shock loading under constant organic loading conditions causes significant changes in steady state yield of cells compared to that under constant concentration conditions. In both cases the yield decreased at decreased dilution rates, and increased at increased dilution rates, but the change was greater under constant organic loading conditions.

4. Under constant concentration conditions generally an increase in flow rate results in a lower protein content, and greater carbohydrate content of cells compared to the respective values before the shock. A reverse situation exists for conditions causing a decrease in flow rate. However, under constant loading conditions any change in flow rate causes an increase in carbohydrate content and decrease in protein content, compared to the values before shock loading.

B. pH Shock Loads

5. Alkaline shock loads up to pH 7.95 do not cause any

severe disruption of purification efficiency in a system operating at neutral pH before the shock.

6. Acid shock loads resulting in reactor pH values of 3.5 or less cause a severe transient state response as shown by high COD values and low biological solids concentrations. Changes in pH resulting in post-shock reactor pH values from neutrality to 5.75 do not cause a serious disruption of purification efficiency. However, the biological solids level drops considerably during the transient state.

7. In all cases of acid shocks in the range of pH from neutrality to 3.0, the purification efficiency is restored after a period of acclimation.

8. As the reactor pH drops from neutrality to the acid range, the predominating microbial species change from bacteria to filamentous types.

9. In all cases the cell yield in the new steady state after a shock load in the acid range of pH was increased; protein content was decreased, and carbohydrate content was increased.

10. Maintenance of a higher level of biological solids by recycle improved the effluent quality during the transient state.

11. Even though acclimated systems operated successfully in the acid range of pH values up to 3.0, the settling properties of the sludge were severely affected, as evidenced in experiments in which cell recycle was practised.

C. Temperature Shock Loads

12. A system operating at a detention time of eight hours and reactor temperature of 25°C can handle temperature shock loads in the range of 17.5°C to 36°C without excessive COD leakage during the transient state, provided the maximum rate of change of temperature was not faster than 1.2°C/hour in the case of a drop in temperature, and 0.6°C/hour in the case of a rise in temperature. Under the same conditions a system at four-hour detention time can accommodate only shock loads in the range 25-36°C. However, even in this case there is considerable decrease in reactor solids level.

13. When the temperature change is not faster than 2°C/hour, systems operating at detention periods of eight and four hours can recover purification efficiency when shock loaded to a temperature of 47°C. However, during the transient state there is considerable COD leakage from the reactor.

14. Large quantities of acetic acid were released as a metabolic intermediate or end product in the system operating at an eight-hour detention time after acclimation was developed during a temperature shock load from 25 to 57.5°C. The acetic acid was utilized when the system reached the new steady state after the shock.

15. After attainment of the final steady state the cell yield and yield of protein and carbohydrate are generally higher at lower temperature, and lower at higher temperature than in the steady state before the shock.

CHAPTER VIII

SUGGESTIONS FOR FUTURE WORK

Based on the results of this investigation, the following suggestions are offered for future work:

1. The response to various combinations of hydraulic and quantitative shock loads may be investigated; e.g., for a given change in flow rate, what change in influent organic concentration may be permitted (or vice versa) to obtain a desired purification efficiency.

2. Using automatic pH control devices, steady state systems may be developed at various reactor pH values, e.g., 4, 5, 6, 8, 9, 10, etc., and shock loaded to pH values above or below that before the shock, and the response patterns may be investigated.

3. The effect of various combinations of hydraulic and pH shock loads on a steady state system may be worth experimentation.

4. The effect of the rate of change of temperature and sludge recycle on temperature shock loads may be studied.

5. The effect of alternating shock loads of cold and hot temperature may be interesting to investigate in basic studies on microbial predominance.

6. The current studies were designed to give an insight

into the general response and magnitude of hydraulic, pH and temperature shock loads which can be accommodated by a completely mixed continuous flow system. Because of the desirability of, and the need for, obtaining the most general pattern of response for these types of shock loadings, each experiment in the current investigation was started from a new heterogeneous seed. It would be of interest, however, to return the system to the previous condition after the shock, to determine whether the population which had been subjected to the various selective pressures imposed by the shock could return to the original steady state.

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